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Problematic Eating Behaviours in Major Depressive Disorder: Links to Peripheral Hormones, Depressive Symptom Profiles and Physical Health Risks

Jessica Grace Mills
University of Wollongong

UNIVERSITY OF WOLLONGONG AUSTRALIA

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Jessica Grace Mills
BPsyc (Hons)

Supervisors:
Dr. Susan Thomas
Dr. Theresa Larkin
Professor Chao Deng

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Graduate Medicine
Faculty of Science, Medicine and Health
University of Wollongong
New South Wales, Australia

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Certification

I, Jessica Grace Mills, hereby declare that this thesis, submitted in partial fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Medicine, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This thesis contains no material which has been submitted or accepted for assessment at any university or equivalent institution. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made.

Signed: J. Mills

Date 12.03.2020

Abstract

Major Depressive Disorder (MDD) is a leading cause of global morbidity and mortality, and is associated with impaired functioning and a poorer quality of life. It is increasingly recognised that individuals with MDD are at greater risk of chronic health conditions such as obesity and cardiometabolic disease, however the pathways linking MDD to chronic disease risk factors are not well understood. An integrated biopsychosocial approach may improve the understanding of the links between MDD and chronic health conditions, potentially leading to more effective treatments. Changes in appetite and weight are symptoms of MDD and may be a pathway between MDD and chronic disease risk factors. Indeed, problematic eating behaviours, such as emotional eating and food addiction, have been linked to depressogenic weight gain. Further, hormones implicated in food intake and eating behaviours such as the satiety hormone leptin, hunger hormone ghrelin, energy balance hormone serotonin and sympathetic stress response hormone dopamine may be dysregulated in MDD. However, the nature of problematic eating behaviours, and whether peripheral hormones are directly related to problematic eating and health indices, has not been systematically studied in MDD. Therefore, this thesis examined relationships between problematic eating behaviours, peripheral hormones, health indices such as body mass index (BMI) and waist circumference, and symptom profiles in MDD to elucidate possible pathways between MDD and chronic health conditions. In **Study 1**, plasma leptin was compared between individuals with MDD (n = 63) and healthy controls (n = 60), by sex and symptom profile. The relationship between leptin and problematic eating was assessed in a subset of individuals with MDD (n = 33). Leptin was higher in females with increased versus decreased or unchanged appetite and weight, with the opposite effect found in males. Problematic eating was more prevalent in depressed females than depressed males, and

positively correlated with leptin concentrations. In **Study 2**, problematic eating, appetite and weight symptoms, and plasma leptin and ghrelin concentrations were examined in a new cohort of individuals with MDD (n = 60) and healthy controls (n = 60). Problematic eating was more prevalent in MDD compared to controls, and in females compared to males. Females had higher leptin than males. Leptin correlated positively and ghrelin negatively with overeating. In the same cohort, **Study 3** examined plasma serotonin in relation to symptom profiles and weight changes in MDD, and relationships between serotonin, problematic eating and depressive symptoms. Plasma serotonin was higher in MDD than controls and in males than females, but did not differ by appetite and weight symptom profile. Serotonin did not correlate with overeating or health indices, however it was positively associated with low mood, negative thinking and insomnia, particularly in males. **Study 4** evaluated plasma dopamine and depressogenic overeating, particularly food addiction, in an extended cohort of individuals with MDD (n = 80) and healthy controls (n = 60) by sex. Participants with MDD who met food addiction criteria had poorer health indices and greater psychopathology than those who did not meet these criteria. Depressed males who met food addiction criteria had lower dopamine than those who did not meet food addiction criteria, whereas dopamine did not vary by food addiction status in females. Dopamine correlated with overeating positively in females and negatively in males. Following the individual assessment of hormones in relation to depressogenic overeating, **Study 5** assessed the extent to which leptin, ghrelin, serotonin and dopamine accounted for unique variance in problematic eating behaviours, depressive symptom severity and BMI in MDD and control participants from Studies 2 to 4 (n = 140), with Sex as a moderating variable. Leptin and dopamine accounted for a significant amount of variance in *Emotional* eating, whereas serotonin accounted for a significant amount of

variance in depressive symptom severity. Leptin and ghrelin further predicted adiposity across sexes, with leptin in particular accounting for a greater amount of variance in BMI in females compared to males. In all studies, problematic eating and hormones correlated with higher BMI and waist circumference values. This thesis provides evidence that individuals with MDD, particularly females, experience greater problematic eating behaviours which are related to peripheral hormones and indices of health risk. The hormonal results suggest that insensitivity or resistance to peripheral hormones, particularly leptin, is significantly associated with overeating in MDD. These findings provide new insight into the nature of depressogenic overeating, and indicate greater hormonal involvement in MDD than previously recognised. These findings present possible avenues for further research into novel and integrated treatments to reduce the risk of chronic health conditions in those affected by MDD, particularly females.

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List of Abbreviations

5-HT	5-hydroxytryptamine
ANOVA	Analysis of Variance
ATQ	Automatic Thoughts Questionnaire
BDI-II	Beck Depression Inventory, Version II
BMI	Body Mass Index
CBT	Cognitive Behavioural Therapy
CIDI	Composite International Diagnostic Interview
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CVD	Cardiovascular Disease
DASS-21	Depression, Anxiety and Stress Scale (21-item version)
DEBQ	Dutch Eating Behaviours Questionnaire
DSM-5	Diagnostics and Statistical Manual of Mental Disorders, Version 5
EDTA	Ethylenediamine Tetra-Acetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FDR	False Discovery Rate
HAM-D	Hamilton Depression Rating Scale
HDRS	Hamilton Depression Rating Scale
HPA	Hypothalamic Pituitary Adrenal
IDS-SR	Inventory of Depressive Symptoms
IHMRI	Illawarra Health and Medical Research Institute
IL-6	Interleukin-6
ISI	Insomnia Severity Index
M	Mean
MDD	Major Depressive Disorder
MINI	Mini International Neuropsychiatric Interview
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
SSRI	Selective Serotonin Reuptake Inhibitor
TNF-α	Tumour Necrosis Factor-alpha
YFAS	Yale Food Addiction Scale

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Publications Arising from Thesis

Full-Length Manuscripts:

Published Articles in Refereed Journals:

Mills, J.G., Thomas, S.J., Larkin, T.A., Pai, N.B. & Deng, C. (2018). Problematic eating behaviours, changes in appetite, and weight gain in Major Depressive Disorder: The role of leptin. *Journal of Affective Disorders, 240,* 137-145. https://doi.org/10.1016/j.jad.2018.07.069.

Mills, J.G., Larkin, T.A., Deng, C. & Thomas, S.J. (2019). Weight gain in Major Depressive Disorder: Linking appetite and disordered eating to leptin and ghrelin. *Psychiatry Research, 279,* 244-251. https://doi.org/10.1016/j.psychres.2019.03.017.

Mills, J.G., Thomas, S.J., Larkin, T.A. & Deng, C. (2020). Overeating and food addiction in Major Depressive Disorder: Links to peripheral dopamine. *Appetite*, *148,* 104586. https://doi.org/10.1016/j.appet.2020.104586.

Articles Submitted for Review in Refereed Journals:

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Abstracts:

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Mills, J.G., Larkin, T.A., Deng, C. & Thomas, S.J. (2018). Relationships between peripheral serotonin and the symptoms of Major Depressive Disorder. *Frontiers in Human Neuroscience*. Conference Abstract: ASP2018 – 28th Annual Conference of the Australasian Society for Psychophysiology. In press.

Mills, J.G., Larkin, T.A., Deng, C. & Thomas, S.J. (2018). Weight gain in Major Depressive Disorder: Linking appetite and eating behaviours to hunger and satiety hormones. *Frontiers in Human Neuroscience*. Conference Abstract: ASP2018 – 28th Annual Conference of the Australasian Society for Psychophysiology. In press.

Mills, J.G., Thomas, S.J., Larkin, T.A. & Deng, C. (2019). Food addiction in Major Depressive Disorder: Relationships to plasma dopamine. *Frontiers in Human Neuroscience*. Conference Abstract: ASP2019 – 29th Annual Conference of the Australasian Society for Psychophysiology. In press.

Note: This thesis is presented as a series of manuscripts prepared for publication. For consistency and structure within the thesis format, all articles are formatted in APA 7th. Further, all subheadings, tables and in-text references to tables have been renumbered to reflect the chapter number in Chapters 2 through to 5. These are the only alterations made to the included manuscripts.

Thesis Overview

Individuals with Major Depressive Disorder (MDD) are at an increased risk of developing chronic health conditions, including obesity and cardiometabolic disorders (Luppino et al., 2010; Penninx, 2017). Despite this risk, the associations between MDD and such conditions are not clearly understood. While MDD is characterised by symptoms spanning psychological and physiological domains, existing approaches in MDD research tend to have a predominantly psychological or physiological focus, which limits comprehensive analyses of the potential factors underlying the relationship between MDD and poor physical health. These approaches also fail to account for the heterogeneous nature of depressive symptom profiles which may further obscure potential pathways (Abreu & Santos, 2008). Biological, psychological and behavioural factors may influence the relationships between MDD, obesity and cardiometabolic disease, however integrated research that combines these factors while accounting for heterogeneity is lacking (Milaneschi et al., 2019).

Weight gain is one of the proposed links between MDD and chronic health conditions. A core symptom of MDD is appetite and weight dysregulation, where individuals can experience either increases or decreases to their appetite and weight (American Psychiatric Association, 2013). Notably, the prevalence of depressogenic weight gain is increasing in concert with the increasing prominence of obesogenic environments (Blanco et al., 2012). Problematic eating behaviours, such as emotional eating and food addiction, may be relevant to weight gain in MDD since such behaviours are risk factors for obesity, which is in turn a risk factor for cardiometabolic disease (Romero-Corral et al., 2010). Additionally, peripheral hormones play a crucial role in eating behaviour by regulating satiety, hunger, weight, energy balance and sympathetic stress, and may therefore also contribute to depressogenic weight gain and

chronic health condition risk (Arora & Anubhuti, 2006). However, problematic eating in MDD is not well understood, and research assessing the relationships between peripheral hormones, eating behaviours and health risk indices in MDD is lacking. Accordingly, this thesis applied an integrated biopsychosocial approach to empirically investigate the nature of problematic eating behaviours in MDD, and how such behaviours relate to psychological measures of depressive symptoms such as appetite and weight change, physical health indices such as BMI and peripheral hormones linked to satiety, hunger, weight regulation and energy balance. These interrelationships were assessed with a view of extending the existing knowledge regarding MDD, obesity and cardiometabolic disease risk. Notably, this is the first program of research to simultaneously examine peripheral hormones, depressive symptom profiles and physical health indices in MDD.

In this thesis research, several types of problematic eating were psychometrically quantified, including emotional eating, restrained eating, external eating and food addiction. The depressive symptom profile of most interest was self-reported appetite and weight gain compared to appetite and weight loss. Other depressive symptoms which may influence problematic eating and weight gain in MDD were also measured, including depressive symptom severity, psychological distress related to mood, anxiety and stress, insomnia and negative thoughts. The physical health indices measured included weight, BMI, waist circumference, blood pressure and heart rate. Peripheral hormones implicated in the regulation of appetite and weight were also examined, which may be relevant to cardiometabolic disturbances and overeating in MDD. Leptin and ghrelin were examined due to their respective roles in satiety and hunger, in addition to energy balance and adiposity. Plasma serotonin was investigated

due to its links to mood, obesity and energy balance, and plasma dopamine was assessed due to its roles in energy balance and the sympathetic stress response.

The opening chapter of this thesis (Chapter 1) provides a comprehensive review of existing literature. The literature review outlines MDD and its symptomology, the chronic conditions that are associated with MDD and the role of weight gain in these conditions. Also outlined in the literature review are potential pathways to depressogenic weight gain, including problematic eating behaviours and the relevant peripheral hormones. Chapter 1 concludes with a statement of the specific thesis aims.

Study 1 (Chapter 2) investigated the role of leptin in appetite and weight changes in MDD (total n = 120; 60 MDD, 60 healthy controls). Problematic eating behaviours, including emotional eating and food addiction, and their links to leptin concentrations were examined in a small subset of the larger study (n = 33). Leptin was linked to appetite and weight change patterns in MDD, which differed by sex, with higher leptin concentrations in depressed females who reported increased appetite and weight, and in depressed males who reported decreased appetite and weight. Leptin correlated positively with problematic eating behaviours in MDD, providing some of the first evidence that problematic eating in MDD is related to a peripheral hormone.

Study 2 (Chapter 3) extended on the previous pilot investigation to compare problematic eating behaviours in MDD compared to controls in a new, larger cohort (total n = 120; 60 MDD, 60 healthy controls). Sex differences and the relationships between leptin, the hunger hormone ghrelin, problematic eating behaviours and appetite/weight changes were also assessed. High levels of problematic eating behaviours were found in MDD, particularly among those who reported appetite and weight gain, and females, supporting that problematic eating behaviours occur frequently in MDD and vary as a function of symptom profile and sex. Leptin correlated

positively, and ghrelin negatively, with problematic eating behaviours and BMI, providing additional evidence that appetite and weight hormones are related to overeating and biometric health indices in MDD.

Having established links between hunger and satiety hormones and problematic eating behaviours in MDD, Study 3 (Chapter 4) examined the energy balance hormone serotonin in MDD compared to controls, in the same cohort as Study 2. Differences in plasma serotonin concentration by sex and appetite/weight changes were measured, and relationships between plasma serotonin, problematic eating behaviours and other depressive symptoms such as low mood, psychological distress, depressogenic thinking and insomnia were assessed. Plasma serotonin was significantly higher in those with MDD than controls, and in males than females. Serotonin correlated with greater depressive symptom severity, depressogenic thinking and anxiety in males, but only with agitation in females. Plasma serotonin did not differ by appetite and weight symptom profile in MDD and was not associated with problematic eating. While serotonin was not directly linked to overeating, it may be an important factor to other symptom profiles in MDD featuring low mood, negative thinking and insomnia disturbances, particularly in males.

Following the high prevalence of problematic eating behaviours identified in participants with MDD relative to controls in Studies 1 and 2, Study 4 (Chapter 5) aimed to assess the extent of overeating and food addiction in MDD, and examine the potential role of peripheral dopamine in these behaviours, in an extended cohort to Studies 2 and 3 (total $n = 140$; 80 MDD, 60 healthy controls). Participants with MDD were more likely to meet the criteria for food addiction than controls, with those meeting criteria for both MDD and food addiction demonstrating greater indices of health risk and psychopathology than depressed individuals who did not meet food

addiction criteria. Plasma dopamine was higher in males compared to females, and correlated positively with problematic eating behaviours in females, and negatively in males. The results of this study provide further support to indicate that problematic eating behaviours in MDD are related to hormones, and also suggests that relationships between plasma dopamine concentrations and overeating behaviours vary by sex.

Following the findings that leptin, ghrelin, serotonin and dopamine were associated with depressogenic problematic eating behaviours and physical health risks in Studies 1-4, Study 5 investigated the combined and individual relative contributions of each of these peripheral hormones to variance in problematic eating behaviours, depressive symptom severity and BMI, and the potential role of Sex in moderating these relationships (total n = 140; 80 MDD, 60 healthy controls). Across sexes, leptin and dopamine predicted problematic eating behaviours, whereas serotonin predicted overall depressive symptom severity. Leptin significantly predicted adiposity as indicated by BMI, with effects being more pronounced in females compared to males. These findings highlight the relative importance of individual hormones in relation to problematic eating behaviours and depressive symptom severity, and that hormones accounted for greater variance in adiposity associated with MDD, as indicated by BMI, in females compared to males.

The final chapter of this thesis (Chapter 7) summarises the overall thesis findings and their collective implications, in addition to discussing the significance of the research. Methodological limitations, directions for future research and general conclusions are also discussed.

CHAPTER ONE

General Introduction and Literature Review

1.1 Major Depressive Disorder

1.1.1 Prevalence

Major Depressive Disorder (MDD) is a multifaceted and heterogeneous mental illness that is a leading cause of morbidity and mortality worldwide, and also the leading contributor to the global disease burden (World Health Organisation, 2017). Approximately 322 million individuals, or 4.5% of the global population, were estimated to be living with MDD in 2017, which affected 5.5% of females and 3.6% of males (World Health Organisation, 2017). In 2018, the 12-month prevalence rate of MDD in Australia was 10.4%, which affected 11.6% of females and 9.1% of males (Australian Bureau of Statistics, 2018). The global prevalence of MDD rose by 18.4% in the decade between 2005 and 2015, with prevalence rates continuing to rise annually (World Health Organisation, 2017). The rising prevalence of MDD has been attributed to increased stress, which can be defined a psychological or physiological response to an experience where situational demands are interpreted to be greater than the resources an individual has to cope (Kogler et al., 2015). These rises are further ascribed to increased endocrine dysregulation, or impaired hormone regulation, as well as an increased shift towards obesogenic lifestyles and environments. Such environments are characterised by increased food intake in response to salient and highly palatable food cues, poor nutrient intake, reduced physical activity and poor physical health (Hidaka, 2012; World Health Organisation, 2017).

1.1.2 Symptoms and Diagnosis

According to the Diagnostics and Statistical Manual of Mental Disorders (DSM-5), MDD is characterised by nine symptoms which broadly span psychological or

physiological domains (American Psychiatric Association, 2013). A core psychological symptom of MDD is *depressed mood*, or a relatively constant negative affective state involving persistent sadness, tearfulness, irritability, pessimism, hopelessness or having a total absence of feeling (Beck et al., 1996). A second core psychological symptom is *anhedonia*, or the impaired ability to experience either pleasure or interest in activities found to be enjoyable in a pre-depressive state such as hobbies, social connections or sexual activities (Treadway & Zald, 2011). Other psychological symptoms include *feelings of guilt or worthlessness* that result from self-blame for minor failings or uncontrollable personal circumstances (McPherson et al., 2007); *executive functioning impairments* including indecisiveness, impaired attention and memory deficits (Paelecke-Habermann et al., 2005); and *suicidal ideation*, or suicidal thoughts that can either be passive without intent to act, or active with intention to see a suicide plan through to completion (American Psychiatric Association, 2013).

Physiological symptoms of MDD often present dichotomously in different symptom profiles. These include *appetite and weight dysregulation*, which presents as either hypophagia and weight loss, or hyperphagia and weight gain (American Psychiatric Association, 2013); *sleep disturbances* of insomnia, which encompasses difficulty falling asleep, difficulty staying asleep or repeatedly waking too early, and hypersomnia, a daily excess of 10 hours of sleep that is often not restful, resulting in increased sleepiness when awake (Geoffroy et al., 2018); and *psychomotor disturbances* presenting as either agitation or retardation, whereby agitation manifests as an inability to sit still, talkativeness or distractibility, and retardation is the slowing of thoughts and behaviours (Sobin et al., 1998). The majority of depressed individuals also exhibit *fatigue,* or lethargy linked to mental or physical exertion (American Psychiatric Association, 2013).

According to DSM-5 criteria, a diagnosis of MDD is based on an individual experiencing at least five out of the nine aforementioned symptoms for a minimum two-week period, termed an 'episode', where one of the five symptoms is required to be either depressed mood or anhedonia (American Psychiatric Association, 2013). Additional diagnostic criteria include self-reported distress, worry or impairment in important areas of an individual's life due to the presence of MDD symptoms, such as in social or occupational environments. Further, the symptoms experienced are required to not be attributable to substance use, a diagnosable medical condition or to another psychological disorder such as anxiety or schizophrenia (American Psychiatric Association, 2013).

1.1.3 Prognosis and Impact

MDD is a recurrent and progressive condition associated with a variable prognosis and course (Parekh et al., 2017). More severe depressive symptoms that are experienced for a longer period of time are linked to a poorer prognosis (Rubenstein et al., 2007). The median duration of a depressive episode is reported to be 24 weeks, however episodes can last from several weeks to several years (Ten Have et al., 2017). Sixty percent (60%) of individuals who experience one depressive episode go on to experience a second within 6 months of recovery from the first. Of these, 80% will experience a third episode, and 90% of those a fourth (American Psychiatric Association, 2013; Burcusa & Iacono, 2007). The highly recurrent nature of MDD can be explained using the 'kindling effect' (Post, 1992), which suggests that an initial depressive episode is triggered by major life stressors, which results in enduring neurochemical brain changes. Subsequent exposure to repeated or prolonged stressors combined with the neurochemical changes results in depressive episodes that are

progressively less linked to specific triggers and more likely to occur without clear cause (Post, 1992). This indicates an increased vulnerability to further depressive episodes as more episodes occur (Post, 1992).

MDD represents a significant physical, emotional and financial burden for those diagnosed, leading to impairment across various domains of inter- and intrapersonal functioning (Hammer-Helmich et al., 2018). Affected individuals often report reduced functional capacity or impairment in social or occupational environments (American Psychiatric Association, 2013), resulting in withdrawal from existing social connections, an inability to form and maintain new social relationships, and a loss of workplace productivity and capacity (Hammer-Helmich et al., 2018). This loss of functioning is associated with a decreased quality of life (IsHak et al., 2015), which is a risk factor for poor treatment outcomes and a greater likelihood of experiencing more recurrent and severe depressive episodes (Hirschfield et al., 2002).

1.2 Obesity and Cardiometabolic Disease Risk and Impact in MDD

A growing body of empirical evidence indicates that those with MDD are at a significantly greater risk of developing chronic health conditions, particularly obesity and cardiometabolic disease (Luppino et al., 2010; Rubin et al., 2013; Steiner et al., 2019). Obesity is also a risk factor for cardiometabolic disease (Romero-Corral et al., 2010). Cardiometabolic disease encompasses cardiovascular and metabolic disorders. Cardiovascular disease (CVD) broadly refers to conditions affecting heart and blood vessel health, including atherosclerosis and myocardial infarction (Penninx, 2017), while metabolic disorders include conditions that feature disturbances to insulin secretion, glucose regulation and lipid abnormalities, including metabolic syndrome (Garcia-Toro et al., 2016). The interaction between MDD and cardiometabolic

conditions appears to be antagonistic with respect to health and functioning (Deschenes et al., 2015; Schmitz et al., 2007). Those with MDD with a cardiometabolic condition are at a significantly greater likelihood of disability, poor social and occupational functioning (Deschenes et al., 2015), morbidity, all-cause mortality (Ofei, 2005; Vaccarino et al., 2008), a reduced quality of life, a poorer prognosis and poor treatment outcomes (IsHak et al., 2015). Indicators of overweight or obesity include high BMI and waist circumference values (Romero-Corral et al., 2010). Risk factors for CVD include high resting heart rates and blood pressure (Gijon-Conde et al., 2015).

There are noted bidirectional relationships between MDD and obesity (Luppino et al., 2010; Mannan et al., 2015). Obesity is characterised by the excessive accumulation of adipose tissue, particularly around the viscera, with this referred to as abdominal adiposity (Ofei, 2005). Clinical and epidemiological studies suggest that MDD increases the risk of obesity by 37-58%, and that obesity increases the risk of MDD by 18-55% (Luppino et al., 2010; Heiskanen et al., 2013; Mannan et al., 2015). MDD is associated with higher BMI and waist circumference values compared to controls (Kloiber et al., 2007; Zhao et al., 2009), with these effects being more pronounced in females than males (Li et al., 2017; Zavala et al., 2018). More severe depressive symptoms are also predictive of greater weight in MDD as indexed by BMI (Heiskanen et al., 2013).

MDD is also associated with an increased risk for developing CVD (Rubin et al., 2013; Penninx, 2017). Depressive symptom severity is a predictor of future CVD onset (Rubin et al., 2013), with a clinical diagnosis of MDD increasing the risk of CVD by approximately 50-90% (Hare et al., 2014). MDD has been associated with high resting heart rates linked to poor emotional regulation (Agelink et al., 2002) and elevated blood pressure (Rubio-Garcia et al., 2013), both of which are associated with

increased sympathetic nervous system activity and increased CVD risk (Nabi et al., 2011). Greater depressive symptom severity has also been correlated with higher blood pressure values (Rubio-Garcia et al., 2013).

1.2.1 Considerations for Understanding the Links Between MDD, Obesity and Cardiometabolic Disease

Despite the increasingly reported associations between MDD, obesity and cardiometabolic disease, the specific pathophysiological pathways between MDD and such conditions are not clear. Some factors which have been implicated in the onset of these conditions in MDD include psychological and physiological stress, unhealthy lifestyles such as increased sedentary activity, changes in diet (Luppino et al., 2010), consistent low-grade inflammation (Knol et al., 2006), weight gain (Rubin et al., 2013), problematic eating behaviours (van Strien et al., 2016) and hormones related to the regulation of food intake (Arora & Ahubhuti, 2006). However, a clear understanding of these mechanisms and associations is complicated by the heterogeneous nature of MDD, as well as a lack of integrated conceptual models for MDD.

1.2.1.1 Heterogeneity in MDD Symptoms

There is substantial heterogeneity in MDD symptom profiles. The requirement of low mood or anhedonia, in addition to another four symptoms, are required for a DSM-5 diagnosis of MDD (American Psychiatric Association, 2013) allows for considerable variability in symptom presentation. Accounting for the dichotomous presentations of appetite/weight dysregulation, sleep disturbances, psychomotor disturbances and suicidal ideation, in addition to any of the other symptoms being present or absent, there are over 227 potential different presentations of MDD that qualify for DSM-5 diagnosis (Zimmerman et al., 2015). There is also a high degree of

variation in treatment responsiveness, as individuals with the same diagnosis but different symptom profiles may respond differently to various treatment types, such as psychological interventions or antidepressant medications (Uher et al., 2011). The cause of this heterogeneity is hypothesised to be a complex interaction between the aetiological factors related to MDD, including changes to the brain and neurochemistry, dysregulation in the hypothalamic-pituitary-adrenal (HPA) axis (Keller et al., 2016), genetics (Lohoff, 2010), environmental factors such as stressful life events or socioeconomic status (Fava & Kendler, 2000), and the various interrelationships between depressive symptoms (Bridwell et al., 2015). Therefore, diverse MDD symptom patterns need to be considered with respect to risk factors for obesity and cardiometabolic disease. However, existing research tends to have combined symptom profiles without consideration of differing presentations. For example, examining weight changes generally without examining the direction of change could mask differences in obesity and cardiometabolic disease risk among particular subgroups of individuals with particular MDD symptom profiles (Fried & Nesse, 2015a).

1.2.1.2 Conceptual Models for MDD

The current dominant models for the pathogenesis and treatment of MDD tend to be divided between those that focus primarily on either psychological factors such as dysfunctional thought patterns, or physiological factors such as dysregulation in brain neurochemistry (Abreu & Santos, 2008; Berton & Nestler, 2006). Psychological models of MDD, including Beck's Cognitive Model (Beck, 1967) suggest that MDD is the by-product of systematic negative biases in thoughts and perceptions. An interaction between early stressful life events and genetic factors is associated with the development of negative cognitive schemas, or stable cognitive frameworks which are

used to interpret the self, the world and the future (Beck, 1967). Negative cognitive schemas are activated in response to biological factors such as stress, and once activated result in a pessimistically distorted perception of reality which contributes to negative mood and behaviour (Beck, 1967). Recurrent activation of these cognitive schemas is associated with an attentional bias towards negative stimuli, consistent low mood and perception, memory and problem-solving deficits (Beck, 1967). Cognitive Behavioural Therapy (CBT; Beck, 1967), developed as a result of Beck's model, is a first line treatment for MDD (RANZCP, 2015). CBT relies on rationalising negative thoughts and replacing such thoughts with more realistic ones through techniques such as monitoring negative thought processes, cognitive restructuring and psychoeducation (Beck, 1967; Powell et al., 2008). CBT has demonstrated efficacy in being able to reduce MDD symptoms after six to twelve therapy sessions (Busch & Sandberg, 2012), however both CBT approaches and Beck's Cognitive Model, while acknowledging a role of genetic factors in the pathogenesis of MDD, have a limited consideration of physiological factors which may be important to MDD. Beck's model was revised in 2008 to outline how biological factors could be incorporated into the existing CBT framework, however this model was largely hypothetical and to date there has been very little research examining the interaction between biological and psychological factors (Beck, 2008; Thomas & Larkin, 2020).

The dominant physiological model of MDD is the monoamine hypothesis, which postulates that low levels of monoamine neurotransmitters in the central nervous system (CNS) are related to depressive symptoms, including serotonin (Charney, 1998). Serotonin has been implicated in the pathogenesis of MDD following the accidental discovery that antipsychotic medications designed to increase serotonergic functioning in schizophrenia were more effective in alleviating depressive than psychosis-related

symptoms (Schildkraut, 1965). The premise of low CNS-serotonin in individuals with MDD led to the development of selective serotonin-reuptake inhibitor (SSRI) medications, where the purported mechanism of action is to block reuptake of serotonin at the neuronal synapses and increase the quantity of serotonin available, with effects on mood (O'Neill et al., 2008). SSRIs have modest efficacy in alleviating symptoms in mild to moderate cases of MDD (Berton & Nestler, 2006), however they are associated with low remission rates, high treatment resistance (Bartolomucci & Leopardi, 2009) and a delayed onset of action. SSRIs are required to be taken for some weeks before effects become evident, resulting in the mechanism of action being questioned, since a quicker response should occur if low CNS-serotonin underlies depressogenic symptoms (Andrews et al., 2015). Notably, the premise of the monoamine hypothesis is difficult to empirically validate, as synaptic levels of serotonin cannot be measured directly (Andrews et al., 2015). As such, CNS-serotonin is commonly estimated in cerebrospinal fluid (CSF) obtained via a lumbar puncture. However, this procedure is highly invasive, requires a large volume of CSF for an accurate reading, and reflects post-neurotransmission serotonin concentrations, which does not indicate original levels in the brain (Andrews et al., 2015; Nishizawa et al., 1997). Similarly to the limitations associated with the psychological models, the monoamine hypothesis can be criticised for a lack of focus on psychosocial factors in the pathogenesis of MDD.

1.2.2 The Need for Integrated Approaches

MDD is a heterogeneous disorder, with diverse psychological and physiological symptoms and aetiological factors. Dominant approaches to conceptualising MDD tend to focus on one potential pathogenesis pathway, with treatment approaches similarly oriented towards addressing either psychological or physiological factors as opposed to

taking an integrated approach (Abreu & Santos, 2008). Considering that MDD consists of both psychological and physiological symptoms, failure to consider both perspectives in combination may result in potential interactions between these factors being overlooked. Examining depressogenic symptom profiles from a biopsychosocial perspective is warranted to provide a more complete understanding of MDD as a disorder, and to develop a clearer account of the associations between MDD, obesity and cardiometabolic diseases (Chen et al., 2000). An integrated approach may allow for the development of tailored intervention methods for depressed individuals at risk of chronic health conditions, in turn improving the prognosis of affected individuals. Such an approach would be advantageous if it can account for unique symptom presentations and pathogenesis factors (Bridwell et al., 2015). However, at present there is a lack of research applying these approaches.

1.3 Pathways between MDD and Cardiometabolic Diseases: Weight Gain

Weight gain, as a precursor to obesity, is a proposed pathway between MDD and cardiometabolic diseases, with increased appetite/weight being the primary depressive symptom explored in the current thesis. A core symptom of MDD is appetite and weight dysregulation, with the direction of appetite/weight changes often varying as a function of MDD subtype (American Psychiatric Association, 2013). MDD has historically been associated with appetite and weight loss, which is symptomatic of melancholia. Melancholic MDD is more prevalent in males than females (Woelfer et al., 2019), and is also characterised by non-reactive mood, blunted emotional responses, pervasive anhedonia, insomnia, loss of libido (American Psychiatric Association, 2013) and HPA axis hyperactivity which is associated with excess cortisol release (Baumeister & Parker, 2012).

However, a growing number of individuals with MDD experience excessive eating and weight gain, which is characteristic of atypical MDD (American Psychiatric Association, 2013). In contrast to melancholic MDD, atypical MDD is more prevalent in females than males (Parker & Thase, 2007; Thase, 2007) and is associated with low mood reactivity, hypersomnia, leaden paralysis, increased sensitivity to interpersonal rejection, excess seeking of interpersonal reassurance (American Psychiatric Association, 2013) and HPA axis hypoactivity featuring a blunted cortisol stress response (Baumeister & Parker, 2012). Individuals with atypical MDD have an elevated risk of weight gain (Lassere et al., 2014), with atypical MDD also associated with higher adiposity and waist circumference values (Jantaratnotai et al., 2016). Epidemiological studies indicate that depressogenic weight gain as part of atypical MDD is now more than 40% more prevalent than weight loss as part of melancholic MDD, likely as result of the increasing prominence of obesogenic diets and environments, and sedentary lifestyles (Blanco et al., 2012). Consistent weight gain and obesity is associated with insulin dysregulation, hypertension, sleep apnoea and high cholesterol, which in turn increase the likelihood of cardiometabolic diseases such as cardiovascular disease and metabolic syndrome (Kearns et al., 2014). Accordingly, individuals with MDD who experience weight gain are at a greater risk of developing these conditions (Kearns et al., 2014; Zhao et al., 2009).

1.3.1 Problematic Eating Behaviours

MDD has been linked to appetite disturbances and associated problematic eating behaviours, which may act as conduits to depressogenic weight gain. Problematic eating behaviours are conceptualised as consisting of emotional eating, restrained eating, external eating and food addiction (Gearhardt et al., 2009, 2016; van Strien et al., 2016).

These problematic eating behaviours can be measured using self-report measures such as the Dutch Eating Behaviours Questionnaire (DEBQ; van Strien et al., 1986) and the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2016).

1.3.1.1 *Emotional Eating*

Emotional eating refers to an increase in food intake in response to negative feelings, which is popularly conceptualised as 'comfort eating' due to its motivation in relieving emotional distress (Finch & Tomiyama, 2015; van Strien et al., 2016). Emotional eating may not be a conscious process, as poor interoceptive awareness of internal satiety signals or physiological distress is associated with emotional eating in response to any arousal state (van Strien & Ouwens, 2007). More severe depressive symptoms have been linked to increased emotional eating (Goossens et al., 2009; Paans et al., 2018; van Strien et al., 2016), particularly the increased intake of carbohydrates and energy dense foods (Christensen & Pettijohn, 2001; Konttinen et al., 2010). In addition, females have higher rates of emotional eating than males (Bailly et al., 2012). As a result of increased food intake, emotional eating is associated with weight gain (Keller & Siegrist, 2015; Porter & Johnson, 2011; van Strien et al., 2016), particularly in atypical MDD (Konttinen et al., 2010).

1.3.1.2 *Restrained Eating*

Restrained eating is the deliberate and conscious restriction of food intake, with the motivation to limit or sustain a preferred body weight (van Strien et al., 2016). Restrained eating behaviours have been reported in MDD (Sevincer et al., 2017), with greater depressive symptom severity associated with greater dietary restraint (Paans et al., 2018). Increased restrained eating behaviours have also been associated with greater anxiety (Appleton & McGowan, 2006) and lower life satisfaction (Remick, Pliner &

McLean, 2009). Higher levels of restrained eating have also been reported in females compared to males (Bailly et al., 2012). Seemingly counterintuitively, restrained eating is often accompanied by binge eating behaviours, which may act as a compensatory response for any psychological or physiological stress experienced as a result of caloric restriction or hunger (Lowe & Kral, 2006). As a result of these compensatory eating behaviours, restrained eating may be associated with higher body weight (van Strien et al., 2016).

1.3.1.3 *External Eating*

External eating is defined as the tendency to selectively attend to food cues, such as the sight and smell of food, resulting in increased food intake (Hou et al., 2011; van Strien et al., 2016). In external eating, food intake is largely regulated by the external food environment, with little influence from internal physiological eating signals such as hunger (Ouwens et al., 2009). Individuals who have a greater tendency to engage in external eating also often have a lower degree of control over these eating behaviours (Heaven et al., 2001; Hou et al., 2011). Increased food intake in response to sensory food cues is a prominent feature of obesogenic environments (Hidaka, 2012). MDD has been linked to external eating in response to highly-palatable-food cues (Ouwens et al., 2009; Sevincer et al., 2017), with the onset of external eating behaviours occurring independently of depressive symptom severity (Paans et al., 2018). More frequent instances of external eating have been related to lower self-esteem (Braet & van Strien, 1997). Similarly to emotional and restrained eating, external eating is also associated with weight gain and obesity as a result of increased food intake (Song, Lee & Sung, 2014).

1.3.1.4 *Food Addiction*

Food addiction is an emerging type of problematic eating behaviour that is defined as the development of addiction-like behaviours associated with the increased intake of highly-palatable foods; particularly those high in sugars or fats (Piccinni et al., 2015). Food addiction is proposed to occur on a continuum of overeating behaviours, ranging from normal eating patterns to addiction (Piccinni et al., 2015). Neuroimaging studies have demonstrated that neural pathways associated with the reward neurotransmitter dopamine are activated in response to highly palatable foods, with repeated exposure to these foods resulting in addiction-like behaviours similar to general substance use disorders such as a greater tolerance to increased food intake and withdrawal when these foods are not consumed (Gearhardt et al., 2009; Gearhardt et al., 2011; Volkow et al., 2012). Food addiction is not currently recognised as a diagnosable disorder; however, criteria have been developed to assess this based on substance use criteria in the DSM-5, including the YFAS (Gearhardt et al., 2016).

The concept of food addiction is currently considered controversial. Food is necessary for survival, whereas the targets of other addictions such as psychoactive substances are not (Onaolapo & Onaolapo, 2018; Ziauddeen & Fletcher, 2013). Similarly, food addiction may also be more appropriately defined as 'additive addiction', as it has been reported that the addictive behaviours occur in response to the sugar or fat content of the foods as opposed to the food as a whole (Onaolapo & Onaolapo, 2018). Many modern foods have been altered to have a higher fat and sugar content compared to naturally occurring foods to increase their palatability (Leigh & Morris, 2018). This in turn increases the likelihood of excessive consumption of these foods and therefore weight gain (Johnson & Wardle, 2014).

Based on YFAS criteria, a 5-10% prevalence of food addiction has been reported in general community samples, and 25% in obese populations (Meule & Gearhardt, 2014). While a prevalence rate of food addiction has not previously been reported in MDD, food addiction has been linked to depressive symptomology. Obese individuals who meet YFAS criteria have greater depressive symptoms than obese controls (Gearhardt et al., 2012). Further, depressive symptoms have been positively associated with the increased consumption of high sugar and fat foods (Crawford et al., 2011; Martins et al., 2019), and the chronic overconsumption of fast food is associated with an increased risk of MDD up to 6 years later (Sanchez-Villegas et al., 2012). It is therefore possible that food addiction may be related to depressogenic weight gain, however this has not yet been evaluated.

1.3.2 Mechanisms Underlying Problematic Eating Behaviours

The motivation to engage in problematic eating behaviours in MDD is widely perceived to be psychologically based. Such problematic eating behaviours, particularly emotional eating and food addiction, may be a coping mechanism used to reduce negative emotions (Finch & Tomiyama, 2015). Despite this conceptualisation, evidence indicates that problematic eating behaviours may occur in response to both psychological and physiological factors, particularly stress (Finch & Tomiyama, 2015; Mouchacca et al., 2013). The physiological stress response is produced by the hypothalamic-pituitary-adrenal (HPA) axis, with elevated HPA axis activity being a common feature of MDD (Keller et al., 2016). The increased preference for highly palatable foods during periods of emotional distress is thought to be a consequence of the ability of some foods to dampen the HPA axis stress response by reducing glucocorticoid activity (Dallman et al., 2003; Hoch et al., 2015). Attenuation of HPA

axis activity facilitates the alleviation or reduction of negative emotions such as low mood, anger or boredom, and their potential replacement by more positive feelings (Gearhardt, et al., 2009). However, evidence indicates that mood-improving effects associated with highly palatable food intake are only temporary (Finch et al., 2019), suggesting that increased food intake in response to emotions may occur to mitigate physiological stress in order to prolong transient mood effects; which may be relevant to the high prevalence of problematic eating behaviours in MDD (Finch et al., 2019). This indicates a potential physiological element to problematic eating behaviours, despite the typical emphasis on its psychological nature only. However, while MDD has been linked to disordered eating (van Strien et al., 2016) and weight gain (Zhao et al., 2009), the psychological and physiological factors involved and relationships between them remain to be elucidated.

1.4 Neuroendocrine Function

Changes to neuroendocrine function, including peripheral hormones, may explain the relationships between MDD, problematic eating behaviours, weight gain and cardiometabolic disease risk. Homeostatic regulation of the endocrine system is essential for optimal mental and physical health (Blevins & Rio, 2013). MDD is associated with prominent neuroendocrine changes, with alterations to neurotransmitter, neuropeptide, thyroid and androgen hormones linked to disturbances in processes such as mood, stress regulation, sleep onset and sleep quality, which are symptoms of MDD (Blevins & Rio, 2013; Chavez-Castillo et al., 2019). The neuroendocrine change most frequently reported and researched in MDD is cortisol dysregulation linked to altered HPA axis activity (Keller et al., 2016). Hypercortisolemia is a risk factor for MDD onset (Herbert, 2012), and individuals with MDD often demonstrate elevated morning

(Keller et al., 2016; Thomas & Larkin, 2018), and wakening (Bhagwagar et al., 2005; Kabia et al., 2015) cortisol concentrations compared to controls, however no differences have also been reported (Steiner et al., 2019). Higher cortisol concentrations are associated with increased stress (Vreeburg et al., 2009), fatigue (Krishnan & Nestler, 2010) and deficits in cognitive functioning (van Londen et al., 1998). In addition, cortisol concentrations are noted to vary as a function of MDD subtype, with hypercortisolemia characteristic of melancholic MDD and hypocortisolemia associated with atypical MDD (Carroll et al., 2007).

However, other peripheral hormones related to the regulation of hunger, satiety, weight, energy balance and the sympathetic stress response, including leptin, ghrelin, serotonin and dopamine, may have key roles in the psychological and physiological mechanisms underpinning eating behaviours, and the regulation of appetite and weight (Arora & Anubhuti, 2006). Changes in the concentrations of these peripheral hormones may be involved in problematic eating behaviours and weight gain in MDD; however, at present there has been little research conducted to test this hypothesis.

1.4.1 Leptin

Leptin is an adipokine hormone secreted by adipocyte cells (Zhang et al., 1994), with significant roles in regulating fat mass and body weight as part of energy homeostasis (Lu, 2007; Maffei et al., 1995). Leptin also has an important anorexigenic influence as it acts as an appetite suppressant during periods of energy excess (Elmquist et al., 1998). While leptin concentrations have been observed to decrease in response to fasting (Nuttall et al., 2016) and increase post-prandially (Korek et al., 2013) as part of the satiety response, previous research comparing leptin in fasted and non-fasted blood samples have identified no overall difference between concentrations (Hancox &

Landhuis, 2011). Notably, non-fasting blood sampling protocols have been utilised in several leptin studies (Ge et al., 2013; Hafner et al., 2012; Rubin et al., 2002), which may reduce the burden on participants (Kearney et al., 2011).

Leptin is secreted in proportion to adipose tissue mass and encourages adipogenesis to account for excess energy intake (Maffei et al., 1995), with plasma leptin concentrations positively associated with BMI and waist circumference values (Chen et al., 2016; Jow et al., 2006; Morris et al., 2012). Subcutaneous administration of leptin is associated with reduced food intake and weight loss (Jow et al., 2006), however the use of leptin as a weight management strategy has not demonstrated long-term success (Heymsfield et al., 1999; Levin & Dunn-Meynell, 2002). This effect may be explained by leptin resistance, a condition where chronically elevated plasma leptin is associated with desensitisation of leptin receptors, resulting in reduced satiety signals, increased food intake and weight gain (Pan et al., 2014). Leptin resistance is a risk factor for obesity, CVD and metabolic syndrome, where it is linked to weight gain (Maffei et al., 1995; Pan et al., 2014) and hypertension (Gijon-Conde et al., 2015).

Leptin dysregulation has been observed in MDD (Antonijevic et al., 1998; Ozsoy et al., 2014; Westling et al., 2004). In particular, elevated leptin concentrations have been reported in individuals with atypical MDD compared to those with melancholia and controls (Gecici et al., 2005; Milaneschi et al., 2017a), suggesting that leptin resistance may be implicated in a subset of individuals with MDD who experience appetite and weight gain. Leptin has also been linked to low mood through its role in regulating HPA axis activity (Hirano et al., 2007; Kim et al., 2006). Leptin secretion is associated with inhibited glucocorticoid production and a suppressed stress adaptation response, which have in turn been linked to greater depressive symptoms (Roubos et al., 2012). Leptin concentrations are sexually dimorphic, with females

exhibiting higher leptin than males (Antonijevic et al., 1998; Rubin et al., 2002) since females naturally store more body fat for reproduction (Blaak, 2001). Despite the associations between leptin, MDD and weight gain, there are no reports on the relationships between leptin in problematic eating behaviours. Given its role in satiety, and potential leptin resistance in atypical MDD, leptin may be related to problematic eating behaviours in MDD.

1.4.2 Ghrelin

Ghrelin is a 28-amino acid hormone secreted from the stomach and gastrointestinal tract (Kojima et al., 1999), with important orexigenic roles in stimulating appetite, and increasing food intake (Wren et al., 2000), adiposity (Tschop et al., 2001) and growth hormone release (Akamizu et al., 2004). Ghrelin exists in two forms: acyl or 'active' ghrelin accounts for approximately 10% of circulating ghrelin concentrations and promotes feeding effects, while desacyl or 'inactive' ghrelin constitutes about 90% of plasma ghrelin and encourages adipogenesis to ensure adequate energy stores during times of energy insufficiency (Patterson et al., 2005; Thompson et al., 2004).

Ghrelin concentrations fluctuate in response to food intake, with higher ghrelin observed prior to eating and reduced levels post-food intake (Akamizu et al., 2004). However, meal-related fluctuations in ghrelin concentrations are noted to occur in both fasted and non-fasted states (Natalucci et al., 2005), suggesting that diurnal rhythms may have a greater influence on ghrelin than fasting status (Nuttall et al., 2016). However, few studies have directly compared ghrelin concentrations in fasted and non-fasted blood samples. Ghrelin is also released during periods of stress, and is implicated in stress-induced weight gain by promoting the intake of highly palatable foods to

mitigate the stress response (Buss et al., 2014). Central and peripheral administration of ghrelin is associated with increased feeding and weight gain (Tschop et al., 2000; Wren et al., 2000), however such feeding and weight changes have not been reported to occur in obese populations (Atalayer et al., 2013). Low ghrelin concentrations have also been associated with risk factors for CVD, particularly high blood pressure and atherosclerosis (Zhang et al., 2010).

Ghrelin dysregulation has been reported in MDD (Barim et al., 2009; Ozsoy et al., 2014), with more severe MDD associated with higher ghrelin concentrations (Algul & Ozcelik, 2017). Ghrelin is also specifically associated with mood (Lutter et al., 2008; Kluge et al., 2011), with one study reporting that 33% of participants reported mood elevations following ghrelin administration (Schmid et al., 2005). Ghrelin is reported to be higher in females compared to males (Soriano-Guillen et al., 2016). However, whether ghrelin concentrations differ as a function of appetite/weight gain or loss in MDD is yet to be determined. Further, the relationship between ghrelin and problematic eating behaviours in MDD has not been examined. Due to its role in food intake, it is possible that ghrelin may be involved in the onset or increased prevalence of problematic eating behaviours in MDD, however similarly to leptin, this has not been assessed in existing ghrelin research.

1.4.3 Serotonin

Serotonin is a monoamine neurotransmitter primarily synthesised by enterochromaffin cells in the GI tract. Peripheral pools of serotonin are separated from central pools in the CNS by the blood brain barrier (Andrews et al., 2015). Impairment of the blood brain barrier has previously been postulated in MDD (Steiner et al., 2011) potentially linked to elevated peripheral serotonin concentrations, which may lead to

crosstalk between the two pools (Maurer-Spurej, 2005). Peripheral serotonin has important roles in modulating GI health, cardiovascular function (Amireault et al., 2013) and metabolic homeostasis (El-Merahbi et al., 2015). Peripheral serotonin also been linked to affect and behaviour regulation via the gut-brain axis, where it acts as a signalling molecule for the vagus nerve afferents that connect the cognitive and affective areas of the brain with the GI tract (O'Mahony et al., 2015; Jenkins et al., 2016). Plasma serotonergic dysregulation has been observed in MDD (Holck et al., 2019; Tyano et al., 2006) and linked to low mood (Messaoud et al., 2018; Paul-Savoie et al., 2011), and these effects reportedly do not differ by sex (Demerdash et al., 2018). There is a lack of research examining peripheral serotonin in relation to other depressive symptoms, including problematic eating behaviours and depressogenic weight gain. In humans, increased carbohydrate intake (Blum et al., 1992) and higher BMI values (Young et al., 2018) have been linked to increased plasma serotonin release. Further, higher plasma serotonin has been associated with metabolic disturbances, increased inflammation, immune activation and pain sensitivity (Shajib & Khan, 2015).

In the CNS, serotonin is implicated in the regulation of mood, sleep, cognition, reward, and HPA axis activity (Berger et al., 2009). Neuroimaging and CSF studies have implicated CNS-serotonin in the control of appetite and food intake (Breum et al., 2003), with elevated concentrations of CNS-serotonin associated with reduced appetite (Kurhe & Mahesh, 2015) and reduced CNS-serotonin related to greater disinhibition with respect to food intake (Bonnet et al., 2017). Serotonergic dysregulation in the CNS has been heavily implicated in the pathogenesis of several psychological disorders, including MDD (Schildkraut, 1965; Jonnakuty & Gragnoli, 2008). Although there are limitations with measuring serotonin directly in the brain (Andrews et al., 2015), studies estimating CNS-serotonin in CSF or using neuroimaging techniques have reported both

lowered (Gao et al., 2008) or elevated (Lemonde et al., 2003) serotonin concentrations in MDD relative to controls. Given that the majority of serotonin is synthesised peripherally, and its links to food intake, weight and chronic health conditions, peripheral serotonin may be related to problematic eating behaviours and weight gain risk in MDD, however these relationships have not yet been examined.

1.4.4 Dopamine

Dopamine is a catecholamine neurotransmitter predominantly synthesised in the periphery by the adrenal medulla where, similarly to peripheral serotonin, peripheral pools are separated from central ones by the blood-brain barrier (Rubi & Maechler, 2010). Peripheral dopamine has important endocrine roles in the homeostatic regulation of blood pressure, plasma glucose concentration, respiration, gastrointestinal motility and circadian rhythms (Rubi & Maechler, 2010). Peripheral dopamine has also been linked to the stress response, with higher levels of physiological stress associated with greater peripheral dopamine release (Rubi & Maechler, 2010), suggesting that dopamine may reflect HPA axis dysregulation in MDD (Dallman et al., 2003). Plasma dopamine dysregulation has been observed in MDD (Fajardo et al., 2003; Pan et al., 2018), and is associated with greater depressive symptom severity (Tomei et al., 2007) and increased psychological stress (Hamner & Diamond, 1996). There is no apparent sexual dimorphism in plasma dopamine concentrations (Fajardo et al., 2003; Pan et al., 2018).

In the CNS, dopamine has important roles in movement, memory and executive function regulation (Drozak & Bryla, 2005). Dopamine is also the primary neurotransmitter implicated in substance use disorders, as it signals hedonic stimuli and motivates individuals to obtain them via neural reward pathways (Treadway & Zald,

2011). In addition to psychoactive substances, dopaminergic reward pathways can be activated in response to highly palatable foods (Meule & Gearhardt, 2014), supporting a possible role for dopamine in the pathogenesis of problematic eating behaviours such as food addiction. CNS-dopamine signals the desire to eat and creates anticipation of reward-driven eating, which is associated with weight gain (Meguid et al., 2000). Hypodopaminergic states in the CNS are associated with low mood and anhedonia in MDD (Belujon & Grace, 2017; Cannon et al., 2009). However, considering that the majority of dopamine is synthesised peripherally and its associations with energy balance and the stress response, it is possible that peripheral dopamine may be related to depressogenic problematic eating behaviours, including food addiction; however, no study to date has examined these factors.

1.5 Summary and Importance

In summary, there are several factors that may be implicated in depressogenic weight gain and therefore cardiometabolic disease risk among individuals with MDD. Previous studies have considered the separate effects of problematic eating behaviours (Paans et al., 2018) or peripheral hormones (Milaneschi et al., 2017a) on depressogenic weight gain, however there is limited research that considers the interrelationships between these factors. This review of the literature indicates that problematic eating behaviours may be related to peripheral hormones including leptin, ghrelin, serotonin and dopamine. While the majority of neuroendocrine research in MDD is oriented on the HPA axis, peripheral hormones related to the regulation of hunger, satiety, weight, energy balance and the sympathetic stress response may be more relevant to understanding problematic eating behaviours and weight changes in MDD. However, there is a lack of research that adequately accounts for the heterogeneity in MDD

symptom profiles, or incorporates psychological and physiological factors into an integrated framework. Examining these relationships from an integrated perspective while accounting for heterogeneity is necessary to better understand the associations between MDD, obesity and cardiometabolic disease, with such findings having the potential to influence how MDD and associated health issues are treated.

Chronic health problems in at-risk individuals have historically been addressed with large-scale and multifaceted public health interventions, such as in the case of smoking and lung-cancer risk. Such interventions, including tax increases on cigarettes, mass-media anti-smoking campaigns, helplines, biological interventions and the implementation of smoke-free zone legislation, have been associated with a substantial reduction in the incidence of smoking and lung cancer diagnoses (Australian Department of Health, 2013). However, despite the risk of weight gain, obesity and cardiometabolic disease in individuals with MDD, there is currently a notable absence of integrated treatment or prevention strategies in the literature for individuals with MDD who are at greater risk of weight gain and health problems in Australia or elsewhere in the world. The responsibility for addressing weight gain is often viewed to be an individual, rather than a public, issue (Brownell et al., 2010), with existing treatment options for depressogenic weight gain including generic weight loss approaches such as dieting, exercise or pharmacotherapy regimes. However, such approaches do not demonstrate long-term success without consistent motivation and maintenance, which is often reduced in MDD (Firth et al., 2016; Jacka & Berk, 2012). There are also considerable social stigmas placing blame for weight gain on an individual's lack of willpower (Brownell et al., 2010). Antidepressant medications, which are commonly prescribed to treat MDD, can also compound the risk of weight gain as this is a common side effect (Moret et al., 2009). The popular perception of

comfort eating and weight gain being psychologically driven is also limiting, as therapeutic approaches may tend to focus on the purported emotional issues inciting an individual to overeat (beyondblue, 2018) while overlooking physiological influences, due to a lack of evidence linking emotional eating to both psychological and physiological factors (Finch & Tomiyama, 2015; Mouchacca et al., 2013). The long-term success of treatments for depressogenic weight gain may also be influenced by peripheral hormones implicated in food intake. Further, the sex differences evident in the incidence of MDD, its subtypes and in peripheral hormones indicate that treatments specific to each sex may also have utility (Smith et al., 2008). Current treatment approaches may be improved if the underlying pathways to depressogenic weight gain are better elucidated, prompting the need for integrated biopsychosocial research to examine these factors. This in turn may result in the increased efficacy of current interventions, and lead to novel preventative measures in order to reduce the likelihood of obesity and cardiometabolic disease in at-risk individuals with MDD.

1.6 Thesis Aims and Hypotheses

The broad aim of the current thesis was to examine the nature of problematic eating behaviours and their relationship to peripheral hormones, health indices and different depressive symptom profiles, in order to better understand the pathways between MDD, obesity and cardiometabolic disease risk. Such research may lead to refined biopsychosocial models of MDD and chronic disease risk, which in turn may lead to improved, tailored interventions for MDD and associated health conditions.

To address these aims, four studies were conducted to investigate associations between problematic eating behaviours, depressive symptoms and symptom profiles, indices of obesity, cardiovascular measures, and peripheral hormones associated with

hunger, satiety, mood and energy balance in MDD. In this thesis, problematic eating behaviours refer to emotional eating, restrained eating and external eating as measured using the DEBQ (van Strien et al., 1986) and food addiction-related symptoms as measured by the YFAS (Gearhardt et al., 2016). Depressogenic appetite and weight changes were defined as increased appetite and weight compared to decreased or unchanged appetite and weight and were determined using the MINI International Neuropsychiatric Interview (Sheehan, 2015). Other depressive symptoms, including low mood, sleep disturbances and negative thinking, were measured using questionnaires such as the Beck Depression Inventory (BDI-II; Beck et al., 1996); Depression, Anxiety and Stress Scale (DASS-21; Lovibond & Lovibond, 1995); Automatic Thoughts Questionnaire (ATQ; Hollon & Kendall, 1980) and the Insomnia Severity Index (ISI; Morin et al., 2009). Obesity indices included BMI and waist circumference values, and cardiovascular measures included blood pressure and heart rate. The peripheral hormones that were quantified were leptin, ghrelin, serotonin and dopamine.

Study 1 examined leptin concentrations in participants with MDD and healthy controls, with its relationships to problematic eating behaviours assessed in a pilot study. Study 2 extended on Study 1 by assessing relationships between leptin, ghrelin and depressogenic problematic eating behaviours and health indices in a larger study. Study 3 assessed the role of peripheral serotonin in relation to depressive symptoms, including problematic eating behaviours and low mood. Study 4 investigated the role of peripheral dopamine in depressogenic problematic eating behaviours and low mood, with a particular emphasis on the concept of food addiction. Finally, Study 5 assessed the overall and unique contribution of leptin, ghrelin, serotonin and dopamine to problematic eating behaviours, depressive symptom severity and BMI.

CHAPTER TWO

2.1 Introductory Comments

This first study examined the relationships between weight changes, problematic eating behaviours and leptin concentrations in MDD. Appetite changes and problematic eating behaviours, including emotional and restrained eating, have been documented in MDD and represent potential pathways to depressogenic weight gain. Leptin may have a role in this pathway, due to its relationship with adiposity and role in satiety. However, there has been no direct assessment of the relationships between leptin, problematic eating behaviours and physiological symptoms of MDD with respect to weight change direction. Understanding these relationships may offer insight into the prevalence of obesity, and consequently cardiometabolic disease risk, in MDD.

Plasma leptin concentrations, biometric measurements including BMI, and psychometric indices of depressive symptoms were compared between 63 individuals with MDD and 60 healthy controls. All participants were further sub-categorised into those with increased, reduced or unchanged appetite/weight for a comparison of the variables of interest by appetite/weight symptom profile. Following anecdotal reports of emotional eating and loss of control around food by some participants in the MDD cohort, the relationships between leptin, biometrics and problematic eating behaviours were examined in a subset of MDD participants as part of a pilot investigation.

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2.2 Abstract

Background: Appetite and weight changes are core symptoms of Major Depressive Disorder (MDD), and those with MDD are at increased risk of obesity, cardiovascular disease and metabolic disorders. Leptin promotes satiety, with leptin dysregulation and resistance noted in obesity. However, the role of leptin in weight changes in MDD is not established. This study investigates leptin levels in relation to appetite and weight changes and problematic eating behaviours in MDD.

Methods: Plasma leptin levels, psychopathology and biometrics were compared between participants meeting DSM-5 diagnostic criteria for MDD (n = 63) and healthy controls (n = 60). Depressed participants were also sub-categorised according to increased, decreased or unchanged appetite and weight. The Dutch Eating Behaviour Questionnaire and Yale Food Addiction Scale were examined in a subset of participants with MDD.

Results: Females with increased appetite/weight had higher leptin levels than those with stable or reduced appetite/weight, however males showed the opposite effect. Leptin levels were positively correlated with problematic eating behaviours. One quarter of the depressed subset, all females, met the Yale criteria for food addiction, approximately double the rates reported in general community samples.

Limitations: The study is limited by a cross sectional design and a small sample size in the subset analysis of eating behaviours.

Conclusions: The results provide new information about associations between leptin, sex-specific weight and appetite changes and problematic eating behaviours, which may be risk factors for cardiovascular and metabolic diseases in MDD, particularly in females. Future longitudinal research investigating leptin as a risk factor for weight gain

in MDD is warranted, and may lead to early interventions aimed at preventing weight gain in at-risk individuals.

Keywords: leptin, Major Depressive Disorder, appetite, weight gain, obesity, food addiction

2.3. Introduction

The global prevalence of Major Depressive Disorder (MDD) is rising annually, with this rise currently being attributed to increasing stress, endocrine dysfunction, modern lifestyle characteristics and dietary patterns (Hidaka, 2012). Altered eating behaviours, and appetite and weight dysregulation, are central diagnostic criteria of MDD (American Psychiatric Association, 2013). MDD is a risk factor for obesity, cardiovascular disease and metabolic syndrome (Cassano & Fava, 2002; Penninx et al., 2013), with a key risk factor being increased appetite; which can occur in MDD (American Psychiatric Association, 2013). Identifying biological and behavioural pathways between MDD and changes in appetite and weight could improve interventions. The hormone leptin is relevant due to its relationship with adiposity and role in satiety, however its relationship to MDD-related weight changes is not properly understood.

There are bidirectional influences between MDD, appetite and weight dysregulation, as MDD increases the risk of becoming obese, and obese individuals are at an increased risk of developing MDD (Kloiber et al., 2007; Luppino et al., 2010). However, depressed individuals may experience either increases or decreases in their appetite and/or weight (Li et al., 2014; Paans et al., 2017); therefore, it is important to consider individual depressive symptom profiles in research relating to health risks. Increased appetite and weight gain are features of atypical MDD, a sub-type of MDD

further characterised by hypersomnia, psychomotor slowing, increased mood reactivity and sensitivity to interpersonal rejection (American Psychiatric Association, 2013). Atypical MDD is further associated with higher body mass index (BMI; Lassere et al., 2014), higher instances of metabolic syndrome (Lamers et al., 2016b) and increased endocrine dysregulation (Gecici et al., 2005; Lamers et al., 2016a; Milaneschi et al., 2017a). There are also indications that weight gain and increased appetite, while previously referred to as 'atypical', are increasingly being identified as a 'typical' symptom of MDD (Privitera et al., 2013). Additionally, a large epidemiological survey indicated that the prevalence of MDD with atypical features was almost 40% higher than that of MDD without atypical features (Blanco et al., 2012). This apparent rise in MDD with weight gain may be associated with the increase of comfort foods in the environment (those high in fat and sugar), which people in emotional distress may seek out (Privitera et al., 2013).

Problematic eating behaviours have also been noted in MDD, which are commonly measured using the Dutch Eating Behaviours Questionnaire (DEBQ; van Strien et al., 1986). These include *Emotional eating*, or increasing food intake in response to negative emotions as a form of coping mechanism; *External eating,* or increased food intake in response to external food cues, including the sight and smell of food; and *Restrained eating,* or deliberately restricting food intake to lose weight or prevent weight gain (van Strien et al., 2016). More severe depressive symptoms are associated with higher emotional eating (van Strien et al., 2016), increased carbohydrate cravings (Christensen & Pettijohn, 2001), increased consumption of energy-dense foods in response to emotional distress (Konttinen et al., 2010) and increased food intake in response to internal and external food cues (Sevincer et al., 2017). Restrictive eating

behaviours, and reduced food intake, have also been linked to depressive symptoms in previous studies (Polivy et al., 1978; Sevincer et al., 2017).

Another approach to understanding overeating is the concept of food addiction, which can be measured using the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2009). It has been proposed that food addiction forms part of a continuum consisting of varying degrees of overeating behaviours that range from normal eating patterns to obesity (Piccinni et al., 2015). Food addiction is also associated with depressed mood, with one study reporting that obese individuals who met the YFAS criteria for food addiction displayed more severe depressive symptoms (Gearhardt et al., 2012). The prevalence of food addiction ranges from 5-10% in the general population, and 15-25% in obese individuals (Meule & Gearhardt, 2014). The preference for palatable foods, including those high in sugar, fat or carbohydrates during periods of emotional distress (Lutter & Nestler, 2009; Hoch et al., 2015) has been attributed to their ability to reduce the psychological and physiological impact of stress (Dallman et al., 2003; Ulrich-Lai, 2016) and activate dopamine-based neural reward pathways (Gearhardt et al., 2011; Volkow et al., 2012), which can result in addiction-like behaviours; similar to alcohol or other psychoactive substances (Gearhardt et al., 2009; Piccinni et al., 2015). Food addiction is not yet recognised as a formal diagnosable disorder and is currently defined as a substance-use disorder in the DSM-5 (American Psychiatric Association, 2013); however, little is known about the relationships between addictive eating behaviours, leptin and MDD.

Leptin is a peptide hormone encoded by the *ob* gene secreted by adipocyte cells, with a critical role in regulating adipose tissue mass and energy balance as part of homeostasis (Lu, 2007). It has a fundamental role as an appetite suppressant during times of excess food consumption (Elmquist et al., 1998; Trayhurn et al., 1999), and is

involved in weight regulation, where plasma leptin levels are released in proportion to adipose tissue mass (Maffei et al., 1995). However, although subcutaneous injection of leptin in lean and obese individuals may lead to decreased food intake and moderate weight loss (Heymsfield et al., 1999), the use of leptin as a long-term weight loss strategy has been unsuccessful (Halaas et al., 1997; Widdowson et al., 1997; Heymsfield et al., 1999; Levin & Dunn-Meynell, 2002). A possible explanation for this is leptin resistance, which is characterised by high leptin levels but decreased leptin sensitivity (Pan et al., 2014). Leptin resistance has been proposed as a mechanism in the pathogenesis of obesity (Zigman & Elmquist, 2003; Ozsoy et al., 2014), with obese animals and humans being noted to have naturally elevated levels of leptin in the absence of food intake (Maffei et al., 1995).

Leptin also moderates stress responses induced by the hypothalamic-pituitary-adrenal (HPA) axis. Leptin secretion has been demonstrated to inhibit corticosteroid production, and thereby suppressing stress adaptation responses (Bornstein et al., 1997; Roubos et al., 2012). Failure of the HPA axis to execute appropriate stress responses may lead to the development of mental disorders such as MDD and anxiety (Blackburn-Munro & Blackburn-Munro, 2001). In animal studies, chronic stress decreases plasma leptin levels (Lu et al., 2006) and insufficient circulating leptin is associated with MDD-like behaviours (Ge et al., 2013; Liu et al., 2017). Administration of leptin to rats after laboratory stressors reversed MDD-like behaviour; suggesting that leptin may also have antidepressant like efficacy (Kim et al., 2006; Hirano et al., 2007). The relationship between leptin and stress, including symptom severity, remains relatively unexplored, particularly in the context of MDD where increased stress is a key feature of the condition (American Psychiatric Association, 2013).

Human studies comparing leptin levels in MDD have found inconsistent results, with either lower (Kraus et al., 2001; Westling et al., 2004; Atmaca et al., 2003) or elevated (Antonijevic et al., 1998; Jiménez et al., 2009; Morris et al., 2012) leptin levels overall between depressed and non-depressed individuals. In contrast, several studies (Häfner et al., 2012; Ozsoy et al., 2014) have identified no difference in leptin levels between depressed and non-depressed individuals; in some cases, even in the presence of appetite loss (Deuschle et al., 1996). No overall difference in leptin levels in individuals with MDD compared to controls was further supported by a recent large-scale meta-analysis by Carvalho et al. (2014), however there were inconsistent results across the studies included. Studies comparing leptin levels across subtypes of MDD have also demonstrated inconsistent results. Individuals with typical/melancholic MDD often demonstrate lower leptin levels compared to controls and individuals with atypical MDD, with the latter demonstrating consistently higher leptin levels overall (Gecici et al., 2005; Lamers et al., 2016a, Milaneschi et al. 2017a). The variability of these results may be due to heterogeneity in appetite and weight change symptoms in MDD, and subsequent alterations in leptin levels. Further, differences in leptin levels between subtypes of MDD with increased or decreased appetite/weight may have been masked in previous research, as symptom subtypes are frequently combined in previous studies.

Further, the methods of classifying atypical MDD symptoms have differed between studies. Some studies have used solely DSM based criteria (Gecici et al., 2005; Lassere et al., 2014), and others have used latent class analysis based on a combination of questionnaire and diagnostic interview responses. The questionnaires and interviews used to measure depressive symptoms for latent class analysis vary, with measures such as the Inventory of Depressive Symptoms (IDS-SR; Rush et al., 1996), Hamilton Depression Rating Scale (HDRS; Hamilton, 1960) and the Composite International

Diagnostic Interview (CIDI; World Health Organisation, 1997) being used (Lamers et al., 2016a, 2016b; Milaneschi et al., 2017a). However, it has been noted that latent class analysis classifications of MDD symptoms do not align completely with DSM-based criteria (Lamers et al., 2016a; 2016b). Therefore, the inconsistent methods may be a potential confounding factor in these studies. Further, not all atypical symptoms have been found to correlate with leptin levels; of the atypical symptoms, changes in weight gain and appetite are most strongly correlated with leptin levels (Lamers et al., 2016a; Milaneschi et al., 2017a). This suggests that changes in appetite/weight may provide a clearer means of subtype classification for the purposes of examining connections between leptin, MDD, appetite and weight gain than the broader criteria of atypical MDD.

Some studies have indicated a sexual dimorphism in leptin levels, with females having higher leptin levels than males (Antonijevic et al., 1998; Rubin et al., 2002). However, other studies have indicated no difference in leptin levels between sexes (Hillemacher et al., 2006; Atmaca et al., 2003). However, there may be interactions between sex and depression status in terms of leptin (Milaneschi et al., 2012; Haleem et al., 2017). Depressed women are more prone to weight gain and obesity than males (Sutin & Zonderman, 2012; Grundy et al., 2014). It is therefore pertinent to investigate leptin levels by sex and MDD status. Further, examining appetite and weight symptom presentations by sex is also important in order to elucidate specific relationships between leptin and MDD symptom profiles.

Due to its role in adiposity, leptin has consistently been correlated with body mass index (BMI) and waist circumference (Jow et al., 2006; Morris et al., 2012; Chen et al., 2016). Leptin has also been related to risk factors for cardiovascular disease, with elevated leptin levels associated with higher systolic and diastolic blood pressure

(Beltowski, 2006; Ma et al., 2009) and heart rate (Brydon, 2011). This is particularly relevant to MDD because of its associations with obesity, cardiovascular disease and metabolic syndrome (Cassano & Fava, 2002; Penninx et al., 2013).

In summary, further research is needed to better understand relationships between leptin levels, eating behaviours and changes in appetite and weight in MDD, and whether these differ by sex. Previous leptin studies have also not measured stress severity, which is relevant considering the role of leptin in moderating activity of the HPA axis. While relationships between leptin, BMI and waist circumference have been previously noted, relationships between leptin and cardiovascular disease risk factors such as blood pressure and heart rate have not yet been sufficiently explored in MDD. Further, while leptin has been linked to depressed mood (Westling et al., 2004; Ozsoy et al., 2014), and depressed mood has been linked to problematic eating behaviours (van Strien et al., 2016; Sevincer et al., 2017), there is no direct research examining the relationships between food addiction and problematic eating behaviours in MDD, particularly in relation to leptin levels. An understanding of these unexplored relationships may elucidate relationships between MDD and other chronic health conditions.

In the current study, plasma leptin levels, biometrics, and psychometric measures of depression, anxiety and stress were compared between individuals with MDD and healthy controls. Depressed participants were sub-categorised to compare those with increased, reduced or unchanged appetite and weight, by sex. Further, relationships between leptin, problematic eating behaviours and food addiction were examined in a subset of depressed participants. In line with previous literature, it was predicted that:

1. Leptin levels would not differ significantly between depressed and non-depressed participants overall.
2. Depressed participants with increased appetite/weight would demonstrate higher leptin levels than depressed participants with decreased or unchanged appetite/weight, with effects being greater in females;
3. Psychometric indices of depression severity, anxiety and stress would positively correlate with leptin levels;
4. Biometric indices of obesity and cardiovascular disease risk factors (including BMI and blood pressure), would positively correlate with plasma leptin levels;
5. Indices of problematic eating and food addiction would positively correlate with leptin levels.

2.4 Methods

2.4.1 Participants

One hundred and twenty-three (123) adults aged between 18 and 69 years (M = 31.87 ± 12.88 years; 70 female, 53 male) participated in the study. 63 participants met the diagnostic criteria for MDD, as confirmed by the Mini International Neuropsychiatric Interview; a valid semi-structured interview based on the DSM-5 designed to assess for psychiatric conditions (MINI; Sheehan et al., 1998; Lecrubier et al., 1997). Participants were recruited by media advertisements, notices and newsletters at the university.

Depressed participants were required to not be receiving any current or recent psychological or medication-based treatment for MDD. The 60 control participants had no history of diagnosed psychiatric disorders. Exclusion criteria across both groups were use of corticosteroids, neurological illness and substance use disorders. Participants were asked to provide details of all medical conditions, medications and

substance use. Eight participants reported a diagnosis of an insulin dysregulation disorder, including diabetes and polycystic ovarian syndrome. No participants had diagnosed eating disorders. No participants were current smokers. The study was approved by the local ethics committee, and all participants provided written informed consent.

2.4.2 Procedure

Data collection occurred at the University Clinical Research Trials Unit. All appointments were scheduled between 9:00am and 11:00am to control for diurnal variations in hormones. On arrival at the clinical trials unit, depressed participants were interviewed using the Mini Neuropsychiatric Interview, version 7.0.2 for DSM 5 (Sheehan, 2015) to confirm MDD diagnoses and symptoms, including weight and appetite changes. Control participants were also asked whether they had experienced recent changes in weight or appetite.

After provision of informed consent, height, weight, blood pressure and heart rate were taken. Waist circumference was measured in the depressed participants only. Following the provision of a non-fasting 10ml blood sample obtained by an experienced phlebotomist, participants then completed the Depression, Anxiety and Stress Scale; a 21 item self-report questionnaire assessing depression, anxiety and stress severity (DASS-21; Lovibond & Lovibond, 1995). The DASS-21 total score has high internal consistency (Cronbach's α = .94; Gloster et al., 2008) The three subscales of the DASS-21 have demonstrated high internal consistency (Cronbach's α = .96 for depression, .89 for anxiety, .93 for stress; Brown et al., 1997).

During the study, when asked about appetite and weight changes, depressed participants frequently volunteered that they experienced loss of control around food

cues and increased emotional eating. Therefore, we obtained ethical approval to administer additional questionnaire measures to the remaining participants, to investigate eating behaviours in more detail, as an exploratory study. The Dutch Eating Behaviours Questionnaire (DEBQ; van Strien et al., 1986) and the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2009) were administered to a subset of 33 depressed participants (20 female, 13 male) in order to investigate eating behaviours in the context of appetite and weight dysregulation in MDD. The DEBQ measures problematic eating styles, including restrained, emotional and external eating behaviours; with each of the three subscales demonstrating high internal consistency (Cronbach's α = .93 for restrained, .93 for emotional, .80 for external eating; van Strien et al., 1986). The YFAS measures food addiction related behaviours, including withdrawal symptoms and loss of control around food cues; with the total YFAS score also demonstrating high internal consistency (Cronbach's α = .86; Gearhardt et al., 2009).

2.4.3 Data and Statistical Analysis

Immediately following blood collection, blood samples were spun in a centrifuge at 4°C, at 2800rpm for 10 minutes. Plasma was immediately stored in a -80°C freezer until analysis. Plasma leptin levels were measured using a standard ELISA (Abcam, Cambridge, United Kingdom) testing kit. The intra- and inter-assay coefficients of the ELISA were <10% and <12% respectively.

Statistical analysis was conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 23). A two-way factorial analysis of variance (ANOVA) was used to test for differences in the leptin levels, with the between subjects factors being Diagnosis (control, depressed) and Sex (male, female), and Age and BMI as covariates. A second two-way factorial ANOVA was used to test for differences in leptin levels as

a function of appetite and weight changes, with the between subjects factors being Appetite and Weight Categories (increased, decreased, no change) and Sex (male, female), with Age and BMI as covariates. Pearson's correlation coefficients and Spearman's rank correlations were used to determine relationships between the variables as appropriate. Post-hoc analyses were conducted using Bonferroni tests. For all statistical tests, $\alpha < .05$ was considered statistically significant.

2.5. Results

2.5.1 Biometric and Psychometric Data

Biometric and psychometric data are displayed in Table 2.1. Diagnostic groups did not differ significantly in age. In terms of sex distributions, there were 35 females in both the MDD and control groups, 25 males in the control group and 28 males in the MDD group. The sex distributions between groups were not significant (χ^2 (2, N = 123) = .097, p = .076).

Systolic blood pressure was higher in males (M = 135.38, SD = 12.92) than females (M = 117.04, SD = 11.64; $F(1, 119) = 68.69$, $p < .001$, partial η^2 =.366). Diastolic blood pressure was also higher in males (M = 80.11, SD = 11.43) than females (M = 75.63, SD = 9.24; $F(1, 119) = 5.52$, p = .020, partial η^2 = .044). No significant effects for Diagnosis or interaction effects were identified for other biometric data.

Depressed participants scored significantly higher on each of the *Depression, Anxiety and Stress* subscales of the DASS compared to controls (Depression: M = 23.21, SD = 9.38 versus M = 6.17, SD = 7.43, $F(1, 119) = 117.59$, $p < .001$, partial η^2 = .497; Anxiety: M = 13.59, SD = 10.65 versus M = 4.60, SD = 5.56, $F(1, 119) = 30.70$, $p < .001$, partial η^2 = .205; Stress: M = 21.90, SD = 9.60 versus M = 10.30, SD = 7.97; $F(1, 119) = 49.99$, $p < .001$, partial η^2 = .296). No significant effects for Sex or interaction effects were identified for DASS scores.

Table 2.1:
Means and standard deviations for biometric and psychometric data, by Diagnosis and Sex (total $N = 123$; MDD and control participants).

		Sex			Diagnosis		
Variable		Male M (SD)	Female M (SD)	Effect *p*	Control M (SD)	Depressed M (SD)	Effect *p*
	Sample Size (*n*)	53	70	-	60	63	-
Biometrics	Age (years)	33.94 (14.23)	30.30 (11.61)	.134	31.83 (10.98)	31.90 (14.55)	.764
	Weight (kg)	84.18 (14.60)	70.15 (17.32)	< .001**	74.53 (16.95)	77.78 (18.16)	.476
	BMI (kg/m^2)	26.52 (4.56)	25.57 (5.85)	.323	25.13 (4.49)	26.77 (5.95)	.147
	Systolic Blood Pressure (mmHg)	135.38 (12.92)	117.04 (11.64)	< .001**	126.40 (14.27)	123.56 (16.01)	.153
	Diastolic Blood Pressure (mmHg)	80.11 (11.43)	75.63 (9.24)	.020*	76.83 (9.86)	78.25 (10.99)	.422
	Heart Rate (bpm)	72.68 (13.68)	73.59 (10.40)	.693	73.29 (12.31)	73.13 (11.53)	.946
Psychometrics	DASS Depression	14.98 (11.55)	14.83 (12.45)	.856	6.17 (7.43)	23.21 (9.38)	< .001**
	DASS Anxiety	8.79 (8.50)	9.51 (10.47)	.580	4.60 (5.56)	13.59 (10.65)	< .001**
	DASS Stress	15.77 (9.81)	16.60 (11.14)	.495	10.30 (7.97)	21.90 (9.60)	< .001**

Note: DASS = Depression, Anxiety and Stress Scale.
[a] * $\alpha < .05$, ** $\alpha < .01$.

2.5.2 Leptin Results

Four participants (2 MDD, 2 controls) did not provide a blood sample, so data from a total of 119 participants were included in the leptin analysis. There were eight univariate outliers in the leptin data detected using boxplot diagrams, with their plasma leptin concentration being greater than two standard deviations above the mean (M = 142.97, SD = 204.48). All outlier values belonged to depressed participants who reported insulin dysregulation disorders. Preliminary statistical analyses were conducted with and without the outliers. These initial results were similar, however when the outliers were removed a higher effect of leptin in MDD overall compared to healthy controls was no longer present. In order to eliminate the possibility of a potential confounding effect of insulin dysregulation on the leptin data, these outliers were excluded from subsequent analyses and results without outliers are henceforth reported.

Preliminary inspection of the distribution of the raw leptin data from the remaining 111 participants indicated a positively skewed distribution (skewness = 3.19, SE = .22). In line with previous human studies (Häfner et al., 2012; Milaneschi et al., 2012) the raw leptin data were natural-log transformed. Means and standard deviations for raw leptin and log-leptin values are displayed in Table 2.2 according to Diagnosis and Sex.

After accounting for age and BMI as potential covariates, log-leptin values did not differ significantly overall between participants with MDD compared to controls ($F(1, 111) = 1.99$, $p = .161$, partial $\eta^2 = .019$). Females ($M = 4.70$, $SD = 1.16$) had significantly higher log leptin values than males ($M = 3.15$, $SD = 1.54$ $F(1, 111) = 81.47$, $p < .001$, partial $\eta^2 = .438$). Age was identified as a significant covariate ($F(1, 111) = 4.43$, $p = .038$, partial $\eta^2 = .040$), as was BMI ($F(1, 111) = 73.86$, $p < .001$, partial $\eta^2 = .413$).

The interaction between Diagnosis and Sex was significant ($F(1, 111) = 4.99$, $p = .028$, partial $\eta^2 = .045$), with depressed males ($M = 3.43$, $SD = 1.40$) having higher log-leptin levels than control males ($M = 2.82$, $SD = 1.66$). Leptin levels did not differ significantly between depressed ($M = 4.86$, $SD = 1.21$) and control females ($M = 4.56$, $SD = 1.12$) overall.

Table 2.2:
Means and standard deviations for raw and log-transformed leptin data, by Diagnosis and Sex (total $N = 111$; MDD and control participants).

Variable		Sample Size	Raw Leptin	Log-Leptin	Main Effect	Diagnosis x Sex Interaction
		n	M (SD)	M (SD)	*p*	*p*
Diagnosis	Control	58	106.31 (104.96)	3.85 (1.61)	.161	.028*
	Depressed	53	149.42 (226.30)	4.14 (1.48)		
Sex	Males	51	54.36 (63.95)	3.15 (1.54)	< .001**	
	Females	60	188.54 (211.59)	4.70 (1.16)		
Covariates	Age (years)	-	-	-	.038*	-
	BMI (kg/m^2)	-	-	-	< .001**	-

Note: significance noted for log-transformed data only.
[a] * $\alpha < .05$, ** $\alpha < .001$.

2.5.3 Analysis by Appetite and Weight Change Groups

The 111 participants were classified according to whether they reported increased ($n = 19$, all depressed), reduced ($n = 20$, all depressed) or unchanged appetite/weight ($n = 72$, 12 depressed, 60 controls) from the clinical interview. No controls reported significant changes in their appetite/weight, whereas participants with MDD reported either increased, decreased or unchanged appetite/weight. One-way ANOVAs indicated no significant differences in biometric variables between appetite/weight presentations (Table 2.3).

Table 2.3:
Means and standard deviations for biometric data, by Appetite/Weight Categories (total $N = 111$; MDD and control participants).

		Increases	Decreases	Unchanged	*p*
		Mean (SD)	Mean (SD)	Mean (SD)	
	Sample Size (*n*)	19	20	72	-
Biometrics	Age	34.95 (17.21)	29.60 (11.53)	32.15 (11.91)	.425
	Weight	79.01 (19.67)	76.00 (15.82)	74.91 (15.88)	.618
	BMI	27.52 (5.75)	25.56 (4.64)	25.30 (4.41)	.174
	Systole	121.95 (15.01)	122.00 (15.41)	126.53 (13.88)	.303
	Diastole	78.35 (9.04)	76.60 (13.69)	77.28 (9.50)	.861
	Heart Rate	73.65 (11.84)	70.15 (9.33)	73.21 (12.19)	.544

[a] Indicates significant differences compared to the 'Increased' group.
* $\alpha < .05$, ** $\alpha < .01$.

The two-way ANOVA with Appetite/Weight categories (increased, decreased, no change) and Sex (male, female) as between group factors, with Age and BMI as covariates (Table 2.4), indicated that leptin values did not differ significantly across appetite/weight presentations ($F(2, 111) = .11$, $p = .900$). However, the interaction between Appetite/Weight categories and Sex was significant ($F(2, 111) = 3.53$, $p = .033$, partial $\eta^2 = .064$). Females with increased ($M = 5.20$, $SD = .87$) or unchanged ($M = 4.64$, $SD = 1.12$) appetite/weight had higher log-leptin values compared to females with decreased appetite/weight ($M = 4.16$, $SD = 1.53$). This pattern was the opposite in males, as those with decreased appetite/weight ($M = 3.65$, $SD = 1.69$) had higher log-leptin values than males with increased ($M = 3.31$, $SD = 0.99$) or unchanged ($M = 2.94$, $SD = 1.56$) appetite/weight.

Leptin values were again significantly higher for females ($M = 4.70$, $SD = 1.16$) compared to males ($M = 3.15$, $SD = 1.54$ $F(1, 111) = 36.90$, $p < .001$, partial $\eta^2 = .264$). BMI was a significant covariate ($F(1, 111) = 69.55$, $p < .001$, partial $\eta^2 = .578$), however age as a covariate was non-significant.

Table 2.4:
Means and standard deviations for raw and log-transformed leptin data, by Appetite/Weight Categories and Sex (total $N = 111$; MDD and control participants).

Variable		Sample Size	Raw Leptin	Log-Leptin	Main Effect	AW x S Interaction
		n	M (SD)	M (SD)	*p*	*p*
Appetite Weight Categories	Increases	19	192.72 (240.58)	4.60 (1.26)		
	Decreases	20	106.31 (156.71)	3.85 (1.60)	.900	
	Unchanged	72	113.16 (156.30)	3.86 (1.58)		.033*
Sex	Males	51	54.36 (63.95)	3.15 (1.54)	< .001**	
	Females	60	188.54 (211.59)	4.70 (1.16)		
Covariates	Age (years)	-	-	-	.133	-
	BMI (kg/m^2)	-	-	-	< .001**	-

Note: significance noted for log-transformed data only.
[a] * $\alpha < .05$, ** $\alpha < .001$.

Weight correlated positively with systolic ($r(111) = .461, p < .001$) and diastolic ($r(111) = .383, p < .001$) blood pressure. BMI also correlated positively with systolic ($r(111) = .257, p = .006$) and diastolic ($r(111) = .390, p < .001$) blood pressure. Further, BMI was positively correlated with weight ($r(111) = .877, p = < .001$). Log-leptin values correlated positively with BMI ($r(111) = .449, p < .001$), but were negatively correlated with systolic blood pressure, ($r(111) = -.240, p = .011$). No significant correlations were identified between log-leptin values, the remaining biometric measurements and DASS subscales.

2.5.4 Subset Analysis of Eating Addiction and Eating Behaviours in MDD

As a further exploratory analysis, waist circumference was measured, and the Dutch Eating Behaviours Questionnaire (DEBQ) and the Yale Food Addiction Scale (YFAS) were administered to 33 participants recruited to the MDD group ($n = 33$). Of these 33 participants, 15 reported increased, 11 decreased and 7 unchanged

appetite/weight. Between-groups analyses based on appetite/weight presentations were not performed due to small cell sizes.

Independent samples *t* tests were performed to investigate Sex differences for the additional measures (Table 2.5). Waist circumference did not differ significantly between sexes ($t(31) = -.64$, $p = .528$, partial $\eta^2 = .013$). Females reported more *Restrained eating* behaviours ($M = 2.83$, $SD = 1.03$) than males on the DEBQ ($M = 1.72$, $SD = .64$; $t(31) = 3.45$, $p = .002$, partial $\eta^2 = .278$). Females also reported more frequent instances of *Emotional eating* ($M = 2.93$, $SD = 1.11$) than males ($M = 2.02$, $SD = .93$; $t(31) = 2.43$, $p = .021$, partial $\eta^2 = .160$). However, *Sensitivity to external food cues* was similar between sexes.

Eight (24%; all female) of the 33 depressed participants in the sub-analysis met the YFAS criteria for food addiction. The *Loss of control* subscale was higher in females ($M = .25$, $SD = .444$) than males ($M = .00$, $SD = .000$; $t(31) = 2.52$, $p = .021$, partial $\eta^2 = .116$). The *Large amounts of time spent acquiring food* subscale was also higher in females ($M = .40$, $SD = .50$) than males ($M = .08$, $SD = .28$; $t(31) = 2.37$, $p = .024$, partial $\eta^2 = .126$). Females reported higher scores than males for *Giving up important activities* for the sake of acquiring food ($M = .45$, $SD = .51$, versus $M = .08$, $SD = .28$; $t(31) = 2.71$, $p = .011$, partial $\eta^2 = .157$) and *Withdrawal symptoms* ($M = .45$, $SD = .51$ versus $M = .08$, $SD = .28$; $t(31) = 2.71$, $p = .011$, partial $\eta^2 = .157$). Females had a higher total number of symptoms on the *Food addiction symptom count* ($M = 3.50$, $SD = 1.82$) than males ($M = 1.92$, $SD = 1.26$; $t(31) = 2.94$, $p = .006$, partial $\eta^2 = .193$). No further differences for the YFAS subscales between sexes were significant.

Table 2.5:
Means, standard deviations and symptom endorsement rates for the eating measures and biometrics in a subset of depressed participants, by Sex (total $N = 33$; MDD only).

Scale		Male	Female	*p*	Endorsement
		Mean (SD)	Mean (SD)		%
	Sample Size (*n*)	13	20	-	-
Biometrics	Waist Circumference (cm)	92.54 (13.70)	88.36 (18.89)	.528	-
DEBQ	Restrained	1.72 (.64)	2.83 (1.03)	.002*	-
	Emotional	2.02 (.93)	2.93 (1.11)	.021*	-
	External Cues	3.03 (.82)	3.34 (.72)	.257	-
YFAS	Loss of Control	.00 (.00)	.25 (.44)	.021*	15.2%
	Inability to Cut Down	1.00 (.00)	.85 (.37)	.083	91%
	Large amounts of Time	.08 (.28)	.40 (.50)	.024*	27.3%
	Giving up Activities	.08 (.28)	.45 (.51)	.011*	30.3%
	Continued Use	.38 (.51)	.50 (.51)	.530	45.5%
	Tolerance	.31 (.48)	.60 (.50)	.107	48.5%
	Withdrawal Symptoms	.08 (.28)	.45 (.51)	.011*	30.3%
	Symptom Count	1.92 (1.26)	3.50 (1.82)	.006*	-

Note: DEBQ = Dutch Eating Behaviours Questionnaire, YFAS = Yale Food Addiction Scale. YFAS subscales are scored dichotomously and occur between 0.00 and 1.00.
* $\alpha < .05$.

Waist circumference was positively correlated with weight ($r(27) = .900, p < .001$), BMI ($r(27) = .874, p < .001$) and diastolic blood pressure ($r(27) = .413, p = .032$). Log-leptin values correlated positively with waist circumference ($r(25) = .510, p = .009$) and BMI ($r(25) = .549, p = .004$), and correlated negatively with heart rate ($r(25) = -.404, p = < .001$). Log-leptin values were also positively correlated with DEBQ *Restrained eating* ($r(25) = .403, p = .046$), and *Emotional eating* ($r(25) = .438, p = .029$). No further results were significant.

2.6. Discussion

This is one of the first studies to examine leptin in relation to increased weight and appetite in MDD. A significant interaction effect between appetite/weight

categories and sex was observed with respect to plasma leptin levels. Females with increased or stable appetite/weight had higher leptin levels than those with reduced appetite/weight; however the opposite effect occurred for males, whereby males with decreased appetite/weight had higher leptin than those with increased or stable appetite/weight. These effects were present with correction for age and BMI. Since leptin has a fundamental role as an appetite suppressant (Elmquist et al., 1998), the higher leptin levels in males with decreased appetite/weight is consistent with normal leptin regulation. However, higher leptin levels in females with increased appetite/weight may be consistent with an interpretation of leptin resistance, a condition which is characterised by chronically elevated leptin levels and decreased leptin sensitivity (Pan et al., 2014). It is possible that the female participants who reported appetite/weight increases have become desensitised to endogenous signals regarding stores of body fat. As such, they continue to experience high levels of hunger despite higher levels of circulating leptin. This may be coupled with continuing to eat as a coping strategy for any physiological and psychological stress they are experiencing, with certain foods dampening HPA activity (Dallman et al., 2003; Ulrich-Lai, 2016).

Leptin levels did not differ significantly between depressed and non-depressed participants in the current study. Further, no significant relationships between leptin levels and depression, anxiety or stress scores were present. Previous research has identified inconsistent results regarding leptin levels in MDD; some studies report increased leptin levels in depressed mood (Antonijevic et al., 1998; Morris et al., 2012), whereas others report lower or equivalent leptin levels in depressed participants versus controls (Carvalho et al., 2014; Westling et al., 2004; Atmaca et al., 2003; Häfner et al., 2012; Ozsoy et al., 2014). Symptom profiles present heterogeneously in MDD, and previous studies did not specifically analyse leptin levels as a function of increased or

decrease weight and appetite. Also, despite previous literature linking leptin to depressed mood (Westling et al., 2004; Morris et al., 2012), the current results, in combination with previous studies, indicate that leptin may be linked to sex-specific symptoms and pathophysiology of MDD (Lu, 2007), as opposed to the disorder as a whole.

A clear overall sex difference in terms of plasma leptin levels was observed in all analyses, with female participants having higher plasma leptin levels than males, irrespective of MDD or appetite/weight change status. This is consistent with previous findings of a sexual dimorphism in leptin studies (Antonijevic et al., 1998; Rubin et al., 2002). Females tend to store more body fat for reproductive purposes (Blaak, 2001) and given a certain BMI, females are likely to have a higher percentage of body fat than males. As leptin secretion is proportional to adipose tissue mass (Maffei et al., 1995), females may have higher circulating leptin levels. Higher leptin levels and body fat percentages may act as a risk factor for weight gain in depressed females, and may perhaps explain why females are more prone to weight gain in MDD (Sutin & Zonderman, 2012; Grundy et al., 2014). Increased weight gain further represents a risk factor for other associated health risks in MDD, including cardiovascular disease and metabolic syndrome (Cassano & Fava, 2002; Penninx et al., 2013). As such, higher leptin levels may also represent a risk factor for these conditions, particularly in depressed females; however further longitudinal research is needed to explore the time course of these factors.

Exploratory analysis of problematic eating behaviours and food addiction in a subset of depressed participants provided promising evidence. To our knowledge, relationships between leptin, MDD, problematic eating behaviours and eating addiction have not been previously reported. In the current study, instances of these problematic

eating behaviours, including emotional and restrained eating, were consistently higher in depressed females compared to depressed males. These results indicate that these behaviours may represent sex-specific coping mechanisms for depressed mood, which are also linked to hormone levels. Further, while depressed mood has previously been associated with increased emotional eating and food intake in response to food cues (van Strien et al., 2016; Sevincer et al., 2017), the current study is the first to identify a direct link between MDD, leptin levels and problematic eating behaviours, including eating addiction. Despite the small sample size for the subset analysis, which limited the power to detect effects, plasma leptin was positively correlated with restrained and emotional eating. This may provide further evidence of leptin resistance in MDD, given that there were higher instances of food consumption and related behaviours despite the presence of equivalent leptin levels. In addition, approximately one quarter of depressed participants in the sub-analysis, all females, met the YFAS criteria for food addition, double the prevalence rates reported in non-clinical samples, and equivalent to that reported in obese samples (Meule & Gearhardt, 2014). This effect relating to food addiction has not, to our knowledge, been previously examined in a depressed sample. This suggests that food addiction may be a more common problem in MDD than previously realised. However, further research is needed to determine whether the role of leptin is causal or is a result of other mechanisms.

Overall, the results of the current study suggest that leptin is related to sex-specific weight changes and problematic eating behaviours in MDD, even after controlling for BMI. Leptin levels were higher in female participants with increased appetite or weight gain, however this pattern was the opposite in males, who showed higher leptin with decreased appetite or weight loss. Leptin levels were positively correlated with measures of comfort eating and loss of control in food consumption.

These results provide further support for leptin dysregulation in problematic eating behaviours in MDD that differs by sex. Leptin resistance may be a factor in appetite and weight dysregulation, particularly in females, and problematic eating behaviours in MDD. Further elucidation of the pathways between depression, health indicators and problematic eating behaviours could assist in the development of early interventions and preventative measures for individuals at risk of weight gain and associated chronic disease due to MDD. Research aimed at identifying interventions to treat leptin resistance is emerging (Pan et al., 2014), which in future may be of value in assisting in reducing the risk of weight gain for depressed individuals.

There are several limitations to the current study. Participants were not required to fast prior to blood collection, and diets were not controlled for. Previous leptin studies have used fasting and non-fasting protocols, and this should be considered when interpreting the results. Additional potential confounding variables, such as physical activity, were not controlled for, and should be considered for future studies. Further, the sub-investigation of food addiction and eating behaviour in relation to leptin was exploratory and conducted for only 33 participants. While these results are promising and showed that problematic eating behaviours were linked to leptin levels, larger studies with control participants are needed to investigate the predictive value of leptin and other hormones in relation to appetite and weight dysregulation. This may further serve as potential modifiable risk factors or points for early intervention in depression or obesity treatment.

In conclusion, the current study provides new insights into the relationships between leptin, problematic eating behaviours, weight gain and MDD. In particular, the results suggest a possible role of leptin resistance in problematic eating behaviours in MDD, particularly in females. This highlights the need for further, longitudinal,

research evaluating the temporal relationships between these variables and the role of leptin and leptin resistance as potential risk factors for weight gain and associated cardiovascular and metabolic health risks in subsets of individuals with MDD. This may lead to opportunities for early interventions aimed at preventing weight gain in at-risk individuals with MDD, and help to address this growing problem.

CHAPTER THREE

3.1 Introductory Comments

The results of Study 1 indicated that problematic eating and weight gain in MDD are related to leptin. The higher leptin concentrations in females with appetite and weight gain, in addition to the positive correlations between leptin and problematic eating behaviours, is suggestive of leptin resistance in MDD and in females. These results indicate that both psychological and physiological variables are linked to problematic eating behaviours, and that integrated approaches may lead to a better understanding of problematic eating in the context of MDD. However, in Study 1 the relationships between leptin and problematic eating were only examined in a small subset of depressed individuals, with no comparisons made to healthy controls. Additionally, ghrelin is implicated in the stimulation of appetite and food intake, but has not been examined in relation to problematic eating and weight gain in MDD.

Therefore, the second study of this thesis expanded on the previous pilot study to examine the relationships between problematic eating behaviours and appetite hormones in a new cohort. Plasma leptin and ghrelin levels, biometrics and psychometric indices of mood and eating were compared between 60 individuals with MDD and 60 healthy controls. A sub-analysis based on self-reported weight changes in the MDD cohort was conducted to assess whether problematic eating and appetite hormones differed based on weight symptom profiles.

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3.2 Abstract

Major Depressive Disorder (MDD) involves changes in appetite and weight, with a subset of individuals at an increased risk of weight gain. Pathways to weight gain may include appetite disturbances, excess eating, and dysregulation of appetite hormones. However, little research has simultaneously examined relationships between hormones, eating behaviours and MDD symptoms. Plasma ghrelin and leptin, biometrics, eating behaviours and psychopathology were compared between depressed (n = 60) and control (n = 60) participants. Depressed participants were subcategorised into those with increased or decreased appetite/weight for comparison by subtype. The Dutch Eating Behaviours Questionnaire and Yale Food Addiction Scale measured eating behaviours. Disordered eating was higher in MDD than controls, in females than males, and in depressed individuals with increased, compared to decreased, appetite/weight. Leptin levels were higher in females only. Leptin levels correlated positively, and ghrelin negatively, with disordered eating. The results provide further evidence for high levels of disordered eating in MDD, particularly in females. The correlations suggest that excessive eating in MDD is significantly linked to appetite hormones, indicating that it involves physiological, rather than purely psychological, factors. Further, longitudinal, research is needed to better understand whether hormonal factors play a causal role in excessive eating in MDD.

Keywords: leptin, ghrelin, depression, obesity, emotional eating, food addiction

3.3. Introduction

Research indicates that obesity increases the risk of Major Depressive Disorder (MDD) by approximately 18-55%, and in turn, MDD increases the risk of obesity by approximately 37-58% (Luppino et al., 2010; Heiskanen et al., 2013; Mannan et al.,

2015). The prevalence rates of both MDD and obesity are increasing annually, with these rises attributed to physiological and psychological stress, obesogenic environments and changes in modern lifestyles (Hidaka, 2012). Consequently, individuals with MDD are at increased risk of developing additional health complications, including cardiovascular disease and metabolic syndrome (Cassano & Fava, 2002; Penninx et al., 2013). Identifying potential mechanisms for weight gain in MDD may help improve interventions aimed at reducing the likelihood of chronic disease in these at-risk individuals.

Changes in appetite and weight are diagnostic criteria for MDD (American Psychiatric Association, 2013), with the direction of appetite/weight changes differing by MDD subtype. Melancholic MDD is characterised by decreased appetite and weight loss. In contrast, atypical MDD features hyperphagia, weight gain (American Psychiatric Association, 2013), higher BMI values, increased endocrine dysregulation and more frequent instances of cardiovascular disease and metabolic syndrome (Gecici et al., 2005; Lamers et al., 2016; Milaneschi et al., 2017a). Epidemiological studies have indicated that atypical MDD is now 40% more prevalent than melancholic MDD; indicating that 'atypical' symptoms, including weight gain, are increasingly becoming more 'typical' (Blanco et al., 2012; Privitera et al., 2013). Despite clearly established risks for chronic disease (Cassano & Fava, 2002; Penninx et al., 2013), there is a lack of both specific treatment guidelines for the treatment or prevention of depressogenic weight gain, and integrated approaches that address both biological and physiological factors. The long-term success of weight loss programs in general is also limited (MacLean et al., 2015). Understanding the mechanisms underlying the pathways to weight gain in MDD may allow for better preventative strategies in individuals at risk due to MDD.

Pathways to weight gain in MDD may include appetite disturbances linked to neuroendocrine changes and associated disordered eating. Disordered eating may act as a coping mechanism for psychological distress, as foods, particularly those high in carbohydrates, can dampen physiological stress responses produced by the hypothalamic-pituitary-adrenal (HPA) axis (Dallman et al., 2003). Disordered eating includes emotional eating, increasing food intake in response to emotional distress; restrained eating, deliberately restricting food intake to prevent weight gain or encourage weight loss; and external eating, increasing food intake in response to sensory food cues (van Strien et al., 2016). Depressed mood and MDD symptom severity are associated with emotional eating (van Strien et al., 2016; Paans et al., 2018), increased consumption of food in response to external cues, and with restricting food intake (Sevincer et al., 2017).

Food addiction characterises a subset of disordered eating behaviours, defined by a preference for highly palatable foods and addiction-like behaviours such as a loss of control and withdrawal (Piccinni et al., 2015). Dopaminergic reward pathways can be activated by highly palatable foods, particularly those high in carbohydrates or fat, which can result in addiction-like behaviours similar to substance use disorders (Gearhardt et al., 2009; Piccinni et al., 2015). Food addiction can be measured using the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2009), with a prevalence of 5-10% in the general population, and 15-25% in obese individuals (Meule & Gearhardt, 2014; Hauck et al., 2017). In a recent pilot, we found that 25% of a sample with MDD met Yale criteria for food addiction, considerably higher than general community samples (Mills et al., 2018).

The concept of food addiction is still controversial, since food is necessary for survival whereas psychoactive substances that form the basis of other addictions are not

(Ziauddeen & Fletcher, 2013: Onaolapo & Onaolapo, 2018). The composition of modern foods has been altered to increase their palatability and hedonistic qualities compared to naturally occurring foods (Leigh & Morris, 2018), to the extent that they could be argued to act similarly to addictive substances. There is increasing evidence of an overlap between activation of neural reward circuitry in response to food and drugs of addiction (Gearhardt et al., 2011; Volkow et al., 2012). Several studies have linked the concept of food addiction to MDD (Gearhardt et al., 2012; Eichen et al., 2013; Mills et al., 2018). As such, it is possible that obesogenic environments promoting the availability of highly palatable foods may be related to the increased prevalence of atypical MDD characterised by weight gain, however further research is needed.

While previous studies indicate that MDD is associated with disordered eating (Bailly et al., 2012; van Strien et al., 2016), food addiction (Eichen et al., 2013; Mills et al., 2018) and weight gain (Blanco et al., 2012), the mechanisms underlying the relationships between these factors are unclear. The hunger and satiety hormones ghrelin and leptin are related to HPA axis activity and suppression of stress responses, and therefore may be associated with MDD and associated symptomatic appetite/weight changes (Roubos et al., 2012; Spencer et al., 2014). However, little research has examined direct relationships between these hormones, disordered eating behaviours and appetite/weight changes in MDD.

Leptin is secreted by adipocyte cells in proportion to adipose tissue mass, with critical roles in regulating adipose tissue and body weight (Maffei et al., 1995). Leptin has important anorexigenic effects, acting as an appetite suppressant during times of energy excess (Elmquist et al., 1998; Trayhurn et al., 1999; Lu, 2007). Leptin resistance, or having high leptin levels but decreased leptin sensitivity (Pan et al.,, 2014) has been implicated in the pathogenesis of obesity (Zigman & Elmquist, 2003; Ozsoy et

al., 2014). Since leptin is secreted relative to adipose tissue mass, higher levels signal the increased availability of fat and in turn lead to a reduction in food intake such that stored fat can be utilised for energy. However, in leptin resistance individuals become desensitised to endogenous satiety signals, resulting in elevated leptin levels without the usual satiety, and subsequently increased food intake and weight gain or obesity (Maffei et al., 1995).

Human research investigating leptin levels in MDD has identified inconsistent results, with either lower (Kraus et al., 2001; Atmaca et al., 2003; Westling, et al., 2004) or elevated (Antonijevic et al., 1998; Jimenez et al., 2009; Morris et al., 2012) leptin levels in MDD versus control populations. In contrast, several studies, including large-scale meta-analyses, have indicated no difference between diagnostic groups (Hafner et al., 2012; Ozsoy et al., 2014; Carvalho et al., 2014). Individuals with atypical MDD have higher leptin levels compared to controls, and individuals with melancholic MDD (Gecici et al., 2005; Lamers et al., 2016a; Milaneschi et al., 2017a), suggesting that leptin may be involved in a subset of individuals with increased appetite/weight.

Ghrelin is secreted from the stomach and gastrointestinal tract (Kojima et al., 1999), with important orexigenic roles in promoting increased food intake (Wren et al., 2000) and increased adiposity (Tschop et al., 2001; Thompson et al., 2004). Significantly lower total ghrelin concentrations have been observed in obese humans, indicating possible downregulation of ghrelin levels associated with excessive eating (Atalayer et al., 2013).

Studies of ghrelin in MDD are also inconsistent, with elevated (Kurt et al., 2008; Atescelik et al., 2017; Ozsoy et al., 2014) lowered (Barim et al., 2009) or no difference between MDD and control participants observed (Kluge et al., 2009; Lawson et al., 2011; Matsuo et al., 2012). Whether ghrelin levels differ by melancholic/atypical

subtype is currently unclear, however due to its role in stimulating appetite levels (Wren et al., 2000) ghrelin levels may possibly be higher in those experiencing appetite/weight gain, or possibly lowered due to the downregulation observed in obesity (Atalayer et al., 2013).

The inconsistent results for leptin and ghrelin levels may be accounted for by the heterogeneity in appetite/weight change symptom profiles in MDD. Previous studies have either combined symptom subtypes (Antonijevic et al., 1998; Ozsoy et al., 2014) or have classified symptoms based on broader atypical MDD criteria, differing also in the classification methods used (Gecici et al., 2005; Lamers et al., 2016; Milaneschi et al., 2017a). These inconsistent classification methods may act as potential confounds in these studies. In addition, leptin levels are more strongly correlated with appetite and weight gain and not other atypical symptoms (Milaneschi et al., 2017a), suggesting that comparison by appetite/weight symptom profile may provide a clearer understanding of relationships between appetite hormones and weight gain than atypical criteria more generally.

Leptin and ghrelin are noted to vary between sexes, with higher leptin and ghrelin levels reported in females than males (Antonijevic et al., 1998; Soriano-Guillen et al., 2016); which may be explained by females having different body fat compositions than males for reproductive purposes (Blaak, 2001). However, sex differences in leptin and ghrelin levels are not always observed (Kluge et al., 2009; Tschop et al., 2001). In humans, leptin and ghrelin levels have also previously been correlated with body mass index (BMI) and waist circumference (Mills et al., 2018; Akamizu et al., 2004), suggesting potential roles for both hormones as risk factors for chronic diseases. However, no studies have simultaneously examined associations between appetite hormones, disordered eating and BMI in MDD.

Our previous pilot study (Mills et al., 2018) found that a high proportion of participants with MDD reported disordered eating, including emotional and restrained eating as measured by the Dutch Eating Behaviours Questionnaire (van Strien et al., 1986), and food addiction behaviours as measured by the Yale Food Addiction Scale (Gearhardt et al., 2009). Disordered eating was higher in females with MDD than males with MDD. Further, disordered eating correlated positively with leptin levels, suggesting that leptin resistance may be involved in disordered eating in MDD. The current study expands on our previous work by investigating eating behaviours in relation to neurobiological measures in a new cohort. Leptin and ghrelin levels, biometrics and psychometric indices of mood and problematic eating behaviours were compared between individuals with MDD and healthy controls. Given the heterogeneous nature of MDD, participants with MDD were further subcategorised by appetite/weight symptom presentation to compare subtypes. It was predicted that:

1. Participants with MDD would report greater levels of disordered eating compared to controls. By symptom profile, MDD participants with increased appetite/weight would demonstrate higher instances of these behaviours than MDD participants without increased appetite/weight; with effects being greater in females.
2. Psychometric indices of problematic eating behaviours, food addiction and depression severity will correlate with leptin and ghrelin levels.
3. Leptin and ghrelin levels would not differ significantly between depressed and non-depressed participants overall. However, at the subgroup level in MDD, these values will be higher in those with increased appetite/weight compared to those with reduced or unchanged appetite/weight; with effects being greater in females.

3.4. Methods

3.4.1 Participants

One hundred and twenty (120) adults aged between 18 and 54 years (M = 25.05, SD = 6.61 years; 68 female) participated. Participants were recruited by media and university advertisements. Depressed participants were pre-screened to confirm that they currently met DSM-5 criteria prior to inclusion in the MDD group (N = 60). Control participants (N = 60) were age and gender matched to the MDD group. Depressed participants were not receiving any current or recent psychological, pharmacological or somatic treatment for MDD. All control participants had no history of diagnosed mental disorders. Use of corticosteroids, neurological illness and substance use were general exclusion criteria. Participants were asked to provide information about any medical conditions and medications being taken. The study received approval from the local ethics committee.

3.4.2 Measures

Depressive symptom severity was assessed using the Beck Depression Inventory (BDI-II), a 21 item self-report questionnaire (Beck et al., 1996). Disordered eating, including *Emotional, Restrained and External* eating, were measured using the Dutch Eating Behaviours Questionnaire (DEBQ; van Strien et al., 1986). Food addiction and related behaviours, including *Withdrawal* symptoms, *Cravings* and *Tolerance* to increased food intake, were assessed using the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2009).

3.4.3 Procedure

Participants attended one visit at the university clinical trials research unit. All participants provided informed written consent. Depressed participants were

interviewed to confirm that they met criteria for MDD using the Mini Neuropsychiatric Interview, version 7.0.2 for DSM-5 (MINI; Sheehan, 2015). Depressed participants were asked to indicate whether they had experienced any recent changes to their appetite or weight. To meet the criteria for increased or decreased/unchanged appetite/weight, MDD participants were required to endorse a 5% (or 3 kilogram) increase or decrease to their weight that was not the result of deliberate weight changes, such as intentional weight loss, in the previous month (Sheehan, 2015).

Participants' height, weight, waist circumference, blood pressure and heart rate were measured, then a 10ml blood sample was obtained by a phlebotomist. All blood samples were taken between 9:00-11:00am. Participants were not required to fast, however details of food consumption in the previous 12h were recorded. Participants then completed the psychometric questionnaires.

3.4.4 Data and Statistical Analysis

Blood samples were centrifuged at 4°C, at 2800rpm for 10 min immediately following blood collection. Plasma was stored in a -80°C freezer until analysis. Plasma leptin and total ghrelin levels were measured using standard ELISA testing kits (Abcam, Cambridge, United Kingdom and Thermofisher Scientific, Carlsbad, United States of America respectively). The intra- and inter-assay coefficients of the leptin ELISA were <10% and <12% respectively, and <6% and <8.5% for the total ghrelin ELISA.

Statistical analysis was conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 23). The dependent variables of interest were the psychometric measurements (BDI, DEBQ and YFAS scores), plasma leptin and total ghrelin levels. Due to the relationship between leptin and adipogenesis (Maffei et al.,

1995), to control for potential confounding effects of visceral fat, plasma leptin was normalised to waist circumference (in metres) prior to statistical analysis.

Two-way factorial analyses of variance (ANOVA) were used to test for differences in the dependent variables with the between-subjects factors of Diagnosis (MDD, control) and Sex (male, female), with Age and BMI used as covariates. Further two-way factorial ANOVAs were used to test for subgroup differences in the variables as a function of appetite and weight changes in MDD participants only, with the between-subjects factors of Appetite and Weight Categories (increased, decreased/no change) and Sex (male, female), with Age and BMI also as covariates. Pearson's correlation coefficients and Spearman's rank correlations were used to determine relationships between the variables. For all statistical tests, $\alpha < .05$ was considered statistically significant. Post-hoc analyses were conducted using Bonferroni corrections.

3.5. Results

3.5.1 Analyses of MDD (combined subtypes) compared to controls)

3.5.1.1 Demographic and Biometric Data

Participant characteristics are shown in Table 3.1. There were 34 females and 26 males in both MDD ($N = 60$) and control ($N = 60$) groups. No participants had a diagnosed eating disorder, and none were current smokers. Of the 120 participants, 30 (18 MDD) had not consumed any food or drink other than water for approximately 12h.

The groups did not differ significantly in age. Males weighed more than females ($F(1, 116) = 18.159$, $p < .001$). No further differences for Diagnosis, Sex or interaction effects were significant for biometric data (Table 3.1).

Table 3.1:
Means and standard deviations for biometric data, by Diagnosis and Sex (total $N = 120$; MDD and control participants).

		Diagnosis				Sex			
Variable		Control M (SD)	MDD M (SD)	Effect *p*	Effect Size partial η^2	Male M (SD)	Female M (SD)	Effect *p*	Effect Size partial η^2
	Sample size (*n*)	60	60	-	-	52	68	-	-
Biometrics	Age (years)	25.40 (7.17)	24.70 (6.03)	.422	.006	25.31 (5.38)	24.85 (7.44)	.709	.001
	Weight (kg)	73.29 (16.67)	74.65 (16.13)	.721	.001	80.79 (13.98)*	68.76 (16.18)	< .001	.135
	BMI (kg/m^2)	25.10 (5.35)	25.80 (5.41)	.580	.003	25.51 (4.35)	25.40 (6.06)	.910	.000
	Waist Circumference (m)	0.89 (0.14)	0.85 (14.30)	.150	.018	0.90 (0.12)	0.82 (0.13)	.071	.028

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index. * Indicates a significant difference compared to the other diagnostic group or sex being compared.

3.5.1.2 Psychometric Data

Participants with MDD had significantly higher Total BDI scores compared to controls ($F(1, 116) = 354.013, p < .001$). Females had higher Total BDI scores than males ($F(1, 116) = 4.860, p = .029$). The interaction between Diagnosis and Sex was not significant.

Depressed participants scored significantly higher on the *Emotional* ($F(1, 116) = 20.194, p < .001$) and *Restrained* ($F(1, 116) = 9.576, p = .002$) subscales of the DEBQ compared to controls. Females also scored significantly higher on the *Emotional* ($F(1, 116) = 10.392, p = .002$) and *Restrained* ($F(1, 116) = 6.268, p = .014$) subscales compared to males. *External* eating did not differ between diagnostic groups or sexes. No interaction effect between Diagnosis and Sex was observed for any DEBQ subscales.

Endorsement rates for each YFAS symptom are presented in Table 3.3. Seventeen (28%; 13 female) MDD participants met the YFAS criteria for food addiction compared to two (3%; both female) of controls. Overall, participants with MDD had significantly more food addiction symptoms than controls ($F(1, 116) = 22.139, p < .001$), and scored significantly higher than controls on each YFAS subscale, with the exception of *Continued Use Despite Problems*. *Withdrawal* scores were significantly higher in females compared to males ($F(1, 116) = 4.208, p = .042$), with no further differences by Sex, and no interaction effects identified (Table 3.2).

Table 3.2:
Means and standard deviations for psychometric data, by Diagnosis and Sex (total $N = 120$; MDD and control participants).

		Diagnosis				Sex			
Psychometric		Control M (SD)	MDD M (SD)	Effect *p*	Effect Size partial η^2	Male M (SD)	Female M (SD)	Effect *p*	Effect Size partial η^2
	Sample size (*n*)	60	60	-	-	52	68	-	-
BDI	Total Score	4.80 (4.75)	30.72 (9.59)*	< .001	.753	16.06 (13.82)	19.06 (15.88)*	.029	.040
DEBQ	Emotional Eating	1.99 (0.72)	2.75 (1.11)*	< .001	.148	2.07 (0.95)	2.60 (.99)*	.002	.082
	Restrained Eating	1.94 (0.83)	2.49 (1.07)*	.002	.076	1.97 (0.93)	2.40 (1.01)*	.014	.051
	External Eating	2.98 (0.71)	3.20 (0.67)	.117	.021	3.08 (0.72)	3.10 (0.69)	.923	.000
YFAS	Increased Intake	0.13 (0.34)	0.33 (0.48)*	.012	.053	0.27 (0.45)	0.21 (0.41)	.410	.006
	Failure to Quit	0.10 (0.30)	0.37 (0.49)*	.001	.093	0.15 (0.36)	0.29 (0.46)	.059	.030
	Time Taken to Obtain	0.10 (0.30)	0.37 (0.49)*	.001	.096	0.27 (0.45)	0.21 (0.41)	.400	.006
	Activities Given Up	0.02 (0.13)	0.28 (0.45)*	< .001	.138	0.13 (0.35)	0.16 (0.37)	.662	.002
	Adverse Consequences	0.07 (0.25)	0.30 (0.46)*	.001	.090	0.15 (0.36)	0.21 (0.41)	.452	.005
	Tolerance	0.03 (0.18)	0.28 (0.45)*	< .001	.111	0.17 (0.38)	0.15 (0.36)	.685	.001
	Withdrawal	0.10 (0.30)	0.42 (0.50)*	< .001	.121	0.17 (0.38)	0.32 (0.47)*	.042	.035
	Use Despite Problems	0.08 (0.28)	0.22 (0.41)	.061	.030	0.10 (0.30)	0.19 (0.40)	.144	.018
	Failed Role Obligations	0.02 (0.13)	0.17 (0.38)*	.006	.063	0.10 (0.30)	0.09 (0.29)	.880	.000
	Physically Hazardous Use	0.08 (0.28)	0.30 (0.46)*	.002	.079	0.21 (0.41)	0.18 (0.38)	.621	.002
	Cravings	0.07 (0.25)	0.23 (0.43)*	.012	.053	0.12 (0.32)	0.18 (0.38)	.348	.008
	Symptom Count	0.80 (3.35)	3.27 (3.45)*	< .001	.160	1.85 (2.89)	2.18 (3.12)	.519	.004

Note: MDD = Major Depressive Disorder; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale. YFAS subscales are scored dichotomously and occur between 0.00 and 1.00. * Indicates a significant difference compared to the other diagnostic group or sex being compared.

Table 3.3:
Endorsement rates for Yale Food Addiction Scale (YFAS) data, by Diagnosis and Sex (total *N* = 120; MDD and control participants).

		Sex		Diagnosis	
		Male	Female	Control	MDD
		%	%	%	%
	Sample size (*n*)	52	68	60	60
YFAS	Increased Intake	26.92	20.59	13.33	33.33
	Inability to Quit	15.38	29.41	10	36.67
	Time Taken to Obtain	26.92	20.59	10	36.67
	Activities Given Up	13.46	16.18	1.67	28.33
	Adverse Consequences	15.38	20.59	6.67	30
	Tolerance	17.30	14.71	3.33	28.33
	Withdrawal	17.30	32.35	10	41.67
	Use Despite Problems	9.61	19.12	8.33	21.67
	Failed Role Obligations	9.61	8.82	1.67	16.67
	Physically Hazardous Use	21.15	17.65	8.33	30
	Cravings	11.54	17.65	6.67	23.33

Note: MDD = Major Depressive Disorder; YFAS = Yale Food Addiction Scale.

3.5.1.3 Leptin and Ghrelin

Initial inspection of the distribution of the waist-circumference normalised leptin data across all participants indicated a positively skewed distribution (skewness = 6.448, *SE* = .231). The normalised leptin data were subsequently natural-log transformed (Milaneschi et al., 2012; Mills et al., 2018). Following log-transformation, three univariate outliers were detected, two of which were MDD participants with leptin levels below the limit of detection. Eleven participants (8 MDD, 3 control; 10 female) had reported insulin dysregulation issues (three had diabetes, two had insulin resistance and six had polycystic ovarian syndrome). In order to eliminate potential confounding effects, the univariate outliers and leptin data from all participants with insulin dysregulation disorders were excluded from subsequent leptin analyses (Mills et al., 2018). Means and standard deviations for the log-transformed leptin values are

displayed in Table 3.4. Log-normalised leptin values did not differ between participants who elected to fast and those who did not ($F(1, 102) = .103, p = .749$).

Accounting for age and BMI, log-normalised leptin values were not significantly different between MDD and control participants. Females had significantly higher log-normalised leptin values than males ($F(1, 100) = 110.391, p < .001$). BMI was a significant covariate ($F(1, 100) = 77.460, p < .001$), however Age as a covariate, and the interaction between Diagnosis and Sex, were non-significant.

Means and standard deviations for the ghrelin data are also displayed in Table 4. Inspection of the ghrelin data indicated a normal distribution (skewness = .135, SE = .231), and no outliers in boxplot diagrams were detected. Participants reporting insulin dysregulation issues ($n = 11$) were also excluded from ghrelin analyses. Ghrelin values did not differ between fasting and non-fasting participants ($F(1, 105) = .164, p = .686$).

Ghrelin values did not differ significantly between MDD and control participants, or by Sex, after accounting for age and BMI as potential covariates. BMI was identified as a significant covariate ($F(1, 103) = 8.007, p = .006$), however Age as a covariate and the interaction between Diagnosis and Sex were non-significant.

Table 3.4:
Means and standard deviations for raw and log-transformed leptin (N = 106) and ghrelin (N = 109) data (ng/ml), by Diagnosis and Sex (MDD and control participants).

Variable			Leptin	Log-Leptin	Main Effect	Effect Size	D x S Interaction		Ghrelin	Main Effect	Effect Size	D x S Interaction
		n	M (SD)	M (SD)	*p*	partial η^2	*p*	*n*	M (SD)	*p*	partial η^2	*p*
Diagnosis	Control	56	8.51 (13.44)	1.11 (1.58)	.173	.018	.814	57	2.77 (1.22)	.994	.000	.668
	MDD	55	16.94 (38.80)	1.42 (1.90)				52	2.70 (1.25)			
Sex	Male	48	3.10 (5.17)	0.13 (1.49)	< .001	.525		51	2.54 (1.10)	.150	.020	
	Female	58	20.26 (36.66)	2.19 (1.32)				58	2.90 (1.31)			
Covariates	Age	-	-	-	.104	.026	-	-	-	.241	.013	-
	BMI	-	-	-	< .001	.436	-	-	-	.006	.072	-

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index. Leptin values normalised to waist circumference. Significance noted for log-transformed data.

3.5.2 Analysis by Appetite/Weight Change Sub-Groups in MDD

All 60 MDD participants were categorised based on self-reported increases (n = 28), decreases (n = 25) or no changes (n = 7) to their appetite/weight from the clinical interview. Data from participants with increased appetite/weight were compared to those with decreased or unchanged appetite/weight (n = 32) combined. Further analyses were performed removing the seven participants with unchanged appetite/weight due to potential differences from participants experiencing appetite/weight dysregulation, however the results were equivalent in terms of significant effects and interactions. Hence, the results from the total sample are reported. There were 17 females and 11 males in the increased group, and 17 females and 15 males in the decreased/unchanged group. The Appetite/Weight Categories did not differ in sex distributions (χ^2 (1, N = 60) = .350, p = .554) or age.

Weight (F(1, 56) = 4.041, p = .049) and BMI (F(1, 56) = 7.480, p = .008) was significantly higher in participants reporting increased appetite/weight than those with decreased/unchanged appetite/weight. Participants with increased appetite/weight also scored significantly higher on the *Emotional* eating subscale of the DEBQ (F(1, 56) = 37.388, p < .001) and reported a greater number of YFAS food addiction symptoms (F(1, 56) = 13.650, p = .001) They also scored significantly higher on each of the YFAS subscales (data not shown). Sex differences for the biometric and psychometric data were as reported previously in sections 3.1.1 and 3.1.2.

Following the exclusion of 10 MDD participants for insulin dysregulation issues as previously described (section 3.3), log-normalised leptin and ghrelin values did not differ between participants who elected to fast and those who did not, and they were not significantly different between those with increased or decreased/unchanged appetite/weight. BMI was a significant covariate in both analyses. Sex effects reflected

those reported previously for the larger comparison (section 3.1.3). No further differences or interaction effects based on Appetite/Weight Categories or Sex were identified. Means and standard deviations for the biometric, psychometric and endocrine data are displayed in Table 3.5.

Table 3.5:
Means and standard deviations for biometric, psychometric ($N = 60$), leptin ($N = 50$; ng/ml) and ghrelin ($N = 52$; ng/ml) data by Appetite/Weight Categories (increased compared to decreased/unchanged) and Sex (MDD participants only).

		Appetite/Weight Categories				Sex			
Variables		Increased	Decreased or Unchanged	Effect	Size	Male	Female	Effect	Size
		M (SD)	M (SD)	*p*	partial η^2	M (SD)	M (SD)	*p*	partial η^2
	Sample Size (*n*)	28	32	-	-	26	34	-	-
Biometrics	Weight (kg)	78.37 (16.6)*	71.4 (15.23)	.049	.067	79.98 (16.48)*	70.58 (14.82)	.014	.104
	BMI (kg/m^2)	27.81 (4.99)*	24.04 (5.20)	.008	.118	25.21 (4.77)	26.25 (5.87)	.580	.006
	Waist Circumference (m)	0.93 (0.14)	0.86 (0.14)	.072	.056	0.90 (0.13)	0.90 (0.15)	.839	.001
BDI	Total Score	31.96 (10.21)	29.62 (9.02)	.444	.011	27.73 (9.44)	33.00 (9.18)*	.041	.072
DEBQ	Emotional Eating	3.49 (0.98)*	2.10 (0.75)	< .001	.400	2.41 (1.12)	3.01 (1.03)*	.024	.087
	Restrained Eating	2.52 (0.97)	2.47 (1.17)	.964	.000	2.17 (1.01)	2.73 (1.07)	.051	.066
	External Eating	3.35 (0.63)	3.08 (0.69)	.087	.051	3.10 (0.73)	3.30 (0.63)	.447	.010
YFAS	Symptom Count	4.96 (3.74)*	1.78 (2.37)	.001	.196	2.81 (3.31)	3.62 (3.57)	.415	.012
Hormones	Leptin (ng/ml)[#]	1.88 (2.00)	1.03 (1.74)	.536	.009	0.24 (1.66)	2.51 (1.40)*	< .001	.506
	Ghrelin (ng/ml)[##]	2.68 (1.39)	2.71 (1.14)	.594	.006	2.48 (1.16)	2.92 (1.31)	.260	.027

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale. [#] N = 50; normalised to waist circumference. [##] N = 52. * Indicates a significant difference compared to the other appetite/weight group or sex being compared.

3.5.3 Correlation Analyses

BMI correlated positively with *Restrained* ($r(120) = .200, p = .028$) and *Emotional* ($r(120) = .274, p = .002$) eating. Waist circumference also correlated positively with *Emotional* eating ($r(120) = .248, p = .006$). YFAS Symptom Count scores correlated positively with participant weight ($r(120) = .217, p = .017$), BMI ($r(120) = .285, p = .002$) and waist circumference ($r(120) = .310, p = .001$). Total BDI scores positively correlated with the *Restrained* ($r(120) = .335, p < .001$) and *Emotional* ($r(120) = .462, p < .001$) subscales of the DEBQ, and with the YFAS Symptom Count score ($r(120) = .501, p < .001$). YFAS Symptom Count scores further correlated with *Restrained* ($r(120) = .216, p = .018$), *Emotional* ($r(120) = .548, p < .001$) and *External* ($r(120) = .211, p = .020$) eating.

Log-normalised leptin values correlated positively with weight ($r(106) = .203, p = .037$), BMI ($r(106) = .490, p < .001$) and waist circumference ($r(106) = .378, p = < .001$). Log-normalised leptin values correlated positively with *Emotional* ($r(106) = .334, p < .001$) and *Restrained* ($r(106) = .366, p = < .001$) eating, and the *Failure to Quit* ($r(106) = .289, p = .003$), *Adverse Consequences* ($r(106) = .198, p = .042$) and *Continued Use Despite Problems* ($r(106) = .218, p = .025$) subscales of the YFAS. Ghrelin values correlated negatively with weight ($r(109) = -.239, p = .012$), BMI ($r(109) = -.247, p = .010$) and waist circumference ($r(109) = -.253, p = .008$) and *Restrained* eating ($r(120) = -.190, p = .048$).

3.6 Discussion

The current study examined relationships between disordered eating, the hunger/satiety hormones ghrelin and leptin and symptomatic weight changes in MDD. It was found that disordered eating was higher in MDD than controls, in females than males, and in a subset of depressed individuals with appetite/weight gain compared to

decreased/unchanged appetite/weight. Leptin levels correlated positively, and ghrelin negatively, with disordered eating.

Emotional and restrained eating behaviours were higher in MDD than controls. Food addiction symptoms were also higher in MDD, with 28% of MDD participants compared to 3% of control participants meeting the Yale criteria for food addiction; double that reported in general community samples (Meule & Gearhardt, 2014) and replicating our earlier findings (Mills et al., 2018). These findings align with previous studies indicating that MDD is associated with increased emotional (van Strien et al., 2016; Paans et al., 2018), restrained (Sevincer et al., 2017) and food addiction eating behaviours (Eichen et al., 2013; Gearhardt et al., 2012). Consistent with our previous study, emotional eating and food addiction were also higher in females compared to males (Mills et al., 2018). In addition, they were higher in participants in the MDD subgroup who had increased compared to reduced or unchanged appetite/weight, consistent with previous research identifying a higher prevalence of these behaviours in overweight and obese populations compared to normal weight controls (Bailly et al., 2012). These findings provide evidence in support of dysregulated appetite and food intake patterns in MDD, particularly in females. Increased intake of highly palatable foods and related behaviours may be used to minimise psychological distress or dampen physiological stress responses produced by the HPA axis (Dallman et al., 2003; Leow et al., 2018), therefore acting as a potential coping mechanism for stress or low mood. This is supported by the positive associations between MDD symptom severity, emotional eating and food addiction. Given that increased food intake and emotional eating have been linked to weight fluctuations (Keller & Siegrist, 2015), these findings provide support for such behaviours acting as potential risk factors for weight gain in those with increased appetite/weight in MDD, particularly females.

Leptin levels were positively correlated with emotional and restrained eating, and food addiction symptoms including loss of control in food consumption and continuing to eat despite negative consequences. Leptin was also positively associated with BMI and weight. The associations between problematic eating behaviours and leptin are consistent with our previous study (Mills et al., 2018), which identified that disordered eating was correlated with leptin levels. Higher leptin levels correlating positively with measures of emotional eating and food addiction relating to increased food intake lead to the suggestion of possible leptin resistance, because higher leptin levels would normally be expected to be associated with satiety rather than behaviours related to increased food intake. In the current study we provide new information about the relationships between ghrelin and some eating behaviours, with ghrelin levels negatively correlating with restrained eating. This suggests that individuals with higher ghrelin levels may be more likely to experience hunger and increased food intake, with a lower degree of control over these behaviours. Comfort eating in MDD is often viewed as having psychological underpinnings (Leow et al., 2018), however the associations between leptin, ghrelin and disordered eating in MDD suggest that excessive eating behaviours are also related to physiological pathways, which is relevant to intervention approaches. Further research is required to assess the temporal nature and causal relationships between these variables.

Leptin and ghrelin levels did not differ significantly by diagnosis, consistent with some previous studies investigating leptin (Carvalho et al., 2014; Mills et al., 2018) and ghrelin (Kluge et al., 2009; Matsuo et al., 2012) levels in MDD, but are in contrast to other studies reporting either elevated or lowered levels (Antonijevic et al., 1998; Ozsoy et al., 2014). The inconsistencies in findings may be due to the heterogeneous nature of symptom profiles in MDD, which has not been a notable factor

in previous research. However, in the current study, neither leptin nor ghrelin levels differed significantly by appetite/weight symptom profile. These findings, in combination with the aforementioned correlations, suggest that leptin and ghrelin may be specifically linked to disordered eating behaviours in MDD as opposed to the disorder more generally, and differences may not be clearly discernible in between-groups analyses.

Clear sex differences were observed in relation to leptin levels, with females having higher waist-normalised leptin levels than males. These results are consistent with previous findings (Antonijevic et al., 1998; Mills et al., 2018). Because females store more body fat for reproductive purposes (Blaak, 2001), and leptin secretion is proportional to adipose tissue mass (Maffei et al., 1995), females may have naturally higher leptin levels. No sex difference was observed in ghrelin levels, consistent with some previous studies (Tschop et al., 2001) but in contrast to others (Soriano-Guillen et al., 2016), indicating that further research is required to examine ghrelin levels in relation to depressogenic weight gain in females also.

These findings help to better understand potential biological, psychological and behavioural pathways associated with depressogenic weight gain, which have previously not been studied in unison. While we did not find an influence of MDD or depression subtypes in leptin levels, their significant correlation with problematic eating behaviours warrants further investigation. Collectively, the identified variables represent promising targets for research into potentially modifiable risk factors for disordered eating behaviours, which may act as risk factors for weight gain in MDD. If increased leptin occurs in the absence of satiety behaviours, but rather in association with depressogenic increased eating corresponding to leptin resistance, this may be a potential therapeutic target to address problematic eating and weight gain risk in a large

subset of individuals with MDD, particularly females. The potential of leptin as a therapeutic target for other symptoms in MDD is currently being considered (Ge et al., 2018), however its potential role in weight gain associated with MDD has received little consideration to date.

There are some limitations of the study which need to be considered. Participants were not required to fast prior to blood sampling; previous leptin and ghrelin studies have used both fasting and non-fasting protocols (Milaneschi et al., 2017a; Mills et al., 2018). Previous research has found that non-fasting leptin levels are not significantly different from fasting leptin levels (Hancox & Landhuis, 2011). Additionally, ghrelin levels maintain characteristic meal-related changes at mealtimes even when fasting (Natalucci et al., 2005). We did compare subgroups of participants who had not consumed food or drink for 12h with those who had and did not find significant hormonal differences, however, use of differing protocols should be considered when interpreting hormone results. Potential confounding variables, such as participants' dietary nutrient intake, physical activity levels and menstrual phase in female participants were also not controlled for. Much previous leptin research does not include information on physical activity or menstrual phase (Ozsoy et al., 2014; Milaneschi et al., 2017a), however these factors should be considered in future studies. This study is also limited by its cross-sectional design. Longitudinal research is necessary to examine temporal relationships, if any, between disordered eating, appetite hormones and weight gain in MDD.

In conclusion, the current study provides new information regarding problematic eating behaviours in MDD and their relationships to hormones and other symptoms. While comfort eating in MDD is often viewed as an emotional issue, the results suggest that self-reported excessive eating in MDD correlates with appetite hormones, implying

that these behaviours may have a greater physiological basis than previously understood, related to appetite and core depressive symptoms. The results also suggest that leptin dysregulation may be important to depressogenic excessive eating and weight gain, representing a potential treatment target for weight gain and associated chronic disease in individuals affected by MDD, particularly females. Future longitudinal research is warranted into these factors.

CHAPTER FOUR

4.1 Introductory Comments

The results of the second study suggested that excessive eating and weight gain in MDD are significantly related to hunger and satiety hormones. These findings suggest that problematic eating behaviours in MDD involve both psychological and physiological processes, further supporting the notion that integrated approaches may be associated with a greater understanding of problematic eating in MDD.

Serotonin has long been implicated in the pathogenesis of MDD, however the role of peripheral serotonin in MDD has not been well investigated. Elevated plasma serotonin has been linked to highly palatable food intake, obesity and energy balance, and may therefore be implicated in depressogenic problematic eating behaviours and appetite/weight changes. However, potential relationships between plasma serotonin, overeating and weight gain have not yet been investigated.

In Study 3, plasma serotonin levels were compared between 60 individuals with MDD and 60 healthy controls, in the same cohort as Study 2. A sub-analysis by appetite and weight changes in MDD was also conducted to determine differences in plasma serotonin levels by this symptom profile. The relationships between plasma serotonin levels, problematic eating behaviours and depressive symptoms related to weight gain, including low mood, depressogenic thinking and sleep disturbances, were also assessed.

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4.2 Abstract

Aims: Individuals with Major Depressive Disorder (MDD) are at an increased risk of chronic disease, partly through appetite and weight gain associated with low mood. Peripheral serotonin has been linked to appetite disturbances and obesity in non-depressed individuals. However, little is known about relationships between plasma serotonin concentrations, specific depressive symptoms, eating behaviours and physical health indicators in MDD.

Methods: Plasma serotonin concentrations and psychopathology were compared between participants with MDD (n = 60) and controls (n = 60) by sex, as well as by symptom-specific subgroups in MDD (increased or decreased/unchanged appetite and weight). Problematic eating behaviours and depressive symptoms were assessed using semi-structured interviews and psychometric questionnaires. Biometric data collected included body mass index and waist circumference.

Results: Plasma serotonin concentrations were significantly higher in participants with MDD than controls, and in males than females. In the total sample, plasma serotonin was associated with depressive symptom severity and insomnia. In males, plasma serotonin was positively correlated with indices of depressed mood, depressogenic thinking and anxiety, whereas in females serotonin was associated with agitation only. Plasma serotonin did not differ by appetite and weight symptom profile in MDD, and was not related to indices of problematic eating behaviours or weight.

Conclusions: Despite no significant links to appetite, weight or physical health indicators, these results provide new evidence to indicate that plasma serotonin levels are meaningfully linked to other symptoms of MDD, including disturbances to mood, cognitions and sleep, particularly in males. These findings suggest that further research into plasma serotonin and its relationships to the symptoms of MDD is warranted.

Keywords: Plasma serotonin; Major Depressive Disorder; symptomology; appetite

4.3. Introduction

Individuals with Major Depressive Disorder (MDD) are at an elevated risk of weight gain (Luppino et al., 2010; Mannan et al., 2015). Appetite and weight changes are a core symptom of MDD and present dichotomously by MDD subtype (American Psychiatric Association, 2013). Hypophagia and weight loss are features of melancholic MDD, whereas hyperphagia and weight gain characterise atypical MDD (American Psychiatric Association, 2013). Importantly, the prevalence of depressogenic weight gain as part of atypical MDD is increasing in concordance with the increasing prominence of obesogenic environments (Blanco et al., 2012; Privitera et al., 2013). Consistent weight gain is associated with a greater risk of chronic conditions such as cardiovascular disease and metabolic syndrome, suggesting that those with MDD who experience weight gain are at a greater risk of these conditions (Kearns et al., 2014; Zhao et al., 2009). However, the pathways between MDD and weight gain are unclear, which is reflected in the lack of effective treatment approaches (Clarke & Currie, 2009). Improved strategies to reduce chronic disease risk in MDD may be developed if the mechanisms underlying depressogenic weight gain are elucidated.

Problematic eating behaviours represent a potential pathway to weight gain in MDD. *Emotional* eating is increasing food intake in response to negative emotions. *Restrained* eating is deliberately restricting food intake to promote weight loss. *External* eating is increasing food intake in response to sensory food cues (van Strien et al., 2016). A fourth type of problematic eating is *food addiction*, that is the development addiction-like behaviours in association with consistent highly palatable food intake (Gearhardt et al., 2016). We previously identified a high prevalence of these behaviours in MDD, particularly in females and in those with appetite and weight gain compared to

loss. Problematic eating behaviours were associated with more severe depressive symptoms, as well as higher BMI and waist circumference values (Mills et al., 2019; Mills et al., 2020). We also identified that these behaviours were related to the satiety hormone leptin (Mills et al., 2018), hunger hormone ghrelin (Mills et al., 2019) and the peripheral sympathetic stress hormone dopamine (Mills et al., 2020), which suggests that depressed individuals may be at a greater risk of weight gain linked to both problematic eating behaviours and biological factors (Mills et al., 2020). In addition to the aforementioned hormones, the peripheral hormone serotonin may also be relevant to problematic eating, weight gain and depressive symptoms in MDD.

Only 5-10% of serotonin originates in the central nervous system (Berger et al., 2009), with the majority of serotonin synthesised peripherally in the gastrointestinal (GI) tract (Wu et al., 2019). Central and peripheral pools of serotonin are separated by the blood brain barrier, and are independently regulated (Andrews et al., 2015). However, it has been suggested that MDD may be associated with greater blood brain permeability (Steiner et al., 2011) that is potentially linked to higher concentrations of serotonin in the periphery, which may lead to crosstalk between the two pools (Maurer-Spurej, 2005). Peripheral serotonin, indexed by plasma or platelet concentrations in blood, is implicated in the regulation of gastric motility, digestion (Amireault et al., 2013), cardiovascular function, inflammation (Wu et al., 2019), energy balance (El-Merahbi et al., 2015) and mood (Jenkins et al., 2016). In addition, plasma serotonin has been linked to food intake and weight gain, with increased carbohydrate intake (Blum et al., 1992) and higher BMI values (Young et al., 2018) associated with higher plasma serotonin. Higher plasma serotonin has also been reported in obese individuals compared to controls (Young et al., 2018), and in chronic health conditions featuring exhaustion and inflammation (Andrews et al., 2015; Shajib & Khan, 2015).

Previous studies investigating plasma serotonin in MDD have identified this to be lower (Messaoud et al., 2018; Paul-Savoie et al., 2011; Sarrias et al., 1987), elevated (Tyano et al., 2006) or not different (Holck et al., 2019; Ortiz et al., 1993; Pan et al., 2018) in MDD compared to controls. Relationships between plasma serotonin and psychopathology, such as depressive symptom severity, are equally inconsistent, with negative (Paul-Savoie et al., 2011; Tyano et al., 2006), positive (Gauthier et al., 2014) or no relationships identified (Messaoud et al., 2018). The inconsistencies between these findings may be a function of differing methodologies and small samples in previous studies, which may limit statistical power and the reliability of the results (Button et al., 2013) or the inclusion of only a composite score of MDD symptoms instead of a symptom level analysis, which may mask effects (Fried & Nesse, 2015b). Plasma serotonin reportedly does not differ by sex (Demerdash et al., 2018), however sex comparisons have not been consistently included in previous studies (Holck et al., 2019; Pan et al., 2018). The mechanisms underlying plasma serotonergic dysregulation in MDD are unclear, however low plasma serotonin may indicate reduced peripheral serotonergic synthesis in MDD (Sarrias et al., 1987). In contrast, due to its links to energy balance and exhaustion, upregulation may reflect a compensatory response for increased physical and emotional energy expenditure as a result of MDD (Andrews et al., 2015). Elevated plasma serotonin prior to antidepressant treatment is also associated with greater antidepressant responsivity, suggesting that higher basal concentrations may have clinical relevance to MDD treatment (Holck et al., 2019).

Given the links to food intake, weight and energy balance, plasma serotonin may be relevant to depressogenic overeating and weight gain, however this is yet to be assessed. Further, to our knowledge, other depressive symptoms, which have the capacity to influence appetite and weight changes in MDD, have not yet been studied in

relation to plasma serotonin. An increased frequency of depressogenic thoughts related to guilt or worthlessness have been linked to greater instances of problematic eating behaviours, which may be used to help cope with such negative thoughts (Conradt et al., 2008). Similarly, sleep disturbances such as insomnia have been linked to appetite increases to compensate for excess energy expenditure (Knutson, 2007). As such, plasma serotonin may indirectly contribute to weight gain risk via psychological effects. Assessing plasma serotonin with respect to appetite and weight and associated symptoms of MDD, with a comparison by symptom profile and sex, may provide a greater understanding of the pathways between MDD and weight gain, which in turn may allow for better preventative measures.

This study aimed to examine plasma serotonin in relation to specific depressogenic symptoms, problematic eating behaviours and weight gain. Plasma serotonin levels were compared between individuals with MDD and healthy controls, by sex. MDD participants were subcategorised into those reporting appetite and weight increases versus decreases, for a comparison of serotonin levels by appetite/weight symptom profile. The relationships between plasma serotonin and low mood, problematic eating behaviours, depressogenic thinking and insomnia were also assessed.

4.4. Methods

4.4.1 Participants

This study was part of a larger investigation examining relationships between mental and physical health in MDD, with the study protocol published elsewhere (Mills et al., 2019). One hundred and twenty (120) adults (52 male) participated in the study, with participants recruited by university and media advertisements. Individuals who were experiencing depressive symptoms (n = 60) were initially screened prior to participation using the MINI Neuropsychiatric Interview (Sheehan, 2015) to confirm

they currently met DSM-5 criteria for MDD. Participants in the MDD group were required to not be receiving any current or recent treatment for MDD in the 2 months prior to participation. Comorbid psychiatric diagnoses were excluded with the exception of generalised anxiety disorder (GAD) due to high comorbidity rates. Sixty (60) control participants were age and sex matched to the MDD group, and had no significant mental health history or diagnosed mental disorders. Exclusion criteria across both groups were use of corticosteroids, neurological illness and substance use disorders. All participants were asked to provide information regarding any medical conditions and any medications being taken. The study was approved by the local joint university and health district ethics committee.

4.4.2 Assessment Instruments

Depressive symptoms were measured using the Beck Depression Inventory (BDI-II; Beck et al., 1996), a 21-item self-report questionnaire that measures symptom severity with cognitive-affective and somatic symptom sub-classifications (Viljoen et al., 2003). The 21-item Depression, Anxiety and Stress Scale (DASS-21; Lovibond & Lovibond, 1995) was used as an index of self-reported psychological distress, and measures depressive, anxiety and stress-related symptomology. Overeating behaviours were assessed using the Dutch Eating Behaviours Questionnaire (DEBQ), a 33-item questionnaire that measures emotional, restrained and external eating behaviours (van Strien et al., 1986). Total food addiction symptoms were measured using the 35-item Yale Food Addiction Scale, version 2 (YFAS; Gearhardt et al., 2016). Additional depressive symptoms, such as depressogenic thinking and insomnia-related sleep disturbances, were respectively assessed using the 30-item Automatic Thoughts Questionnaire (ATQ; Hollon & Kendall, 1980) and the summed score of the first three items of the Insomnia Severity Index (ISI; Morin et al., 2011).

4.4.3 Procedure

Participants attended one visit at the university Clinical Research and Trials Unit. All participants provided written informed consent. Participants with MDD were interviewed with the Mini Neuropsychiatric Interview, version 7.0.2 for DSM-5 (MINI; Sheehan, 2015) to confirm that at the time of their visit they met the criteria for a current depressive episode. Participants' height and weight were measured to calculate body mass index (BMI) values, and waist circumference measurements were also taken. A 10ml blood sample was then collected in an EDTA coated tube by a phlebotomist, with all blood samples collected between 9:00-11:00am to control for diurnal variations in hormones. Participants were not required to fast, however details of food intake in the 12 hours preceding the blood sample were recorded. Participants then completed the psychometric questionnaires.

4.4.4 Data and Statistical Analysis

4.4.4.1 Blood and Serotonin Analyses

Immediately following collection, 20µl per millilitre of aprotinin inhibitor was added to each blood sample to prevent blood coagulation. Blood samples were then centrifuged at 4°C, at 2800rpm for 10 minutes. Aliquoted plasma was stored at -80°C until analysis. Plasma serotonin concentrations were quantified measured using standard ELISA kits (Abcam, Cambridge, United Kingdom). The ELISA inter- and intra-assay coefficients were <16% and <5% respectively.

4.4.4.2 Statistical Analyses

Statistical analyses were conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 25). The dependent variables of interest were plasma serotonin concentrations, the biometric measurements (BMI, waist circumference) and

the psychometric measurements (BDI-II, DASS, DEBQ, YFAS, ATQ and ISI). The questionnaire values were log-transformed to correct for skewed distributions, however the results for transformed and untransformed data were equivalent in terms of significant effects and interactions; hence, the results from untransformed analyses only are reported.

Two-way factorial analyses of variance (ANOVA) were used to compare groups on the psychometric and biological measures, with between-subjects factors of Diagnosis (MDD, control) and Sex (female, male), and Age entered as a covariate. Two-way ANOVAs were also used to determine subgroup differences in the dependent variables as a function of appetite and weight changes in MDD participants only, with the between-subjects factors of Appetite and Weight Change (increased appetite/weight, decreased/unchanged appetite/weight) and Sex (female, male), and Age as a covariate. An $\alpha < .05$ was considered statistically significant.

Pearson's correlation coefficients were used to determine relationships between the study variables. To reduce the probability of Type I errors in the correlational analyses, the false discovery rate (FDR) control procedure (Benjamini & Hochberg, 1995) was applied. FDR-corrected *p* values, with an $\alpha < .05$, are reported for all *r* statistics.

4.5. Results

4.5.1 Comparisons between MDD and controls

4.5.1.1 Biometric and Psychometric Data

Demographic and biometric data for the total sample are presented in Table 4.1. Participants were aged between 18 and 54 years ($M = 25.05$, $SD = 6.61$ years), with 34 females and 26 males in both MDD ($n = 60$) and control ($n = 60$) groups. No participants had a diagnosed eating disorder, and none were smokers. The diagnostic

groups (MDD versus control) did not differ significantly in age. As previously reported, males weighed more than females, with no further differences by Diagnosis, Sex or interaction effects identified for biometric data (Mills et al., 2019).

Means and standard deviations for psychometric data are also presented in Table 4.1. Participants with MDD had significantly higher BDI *Cognitive* and *Somatic,* DASS *Depression, Anxiety* and *Stress*, ATQ and ISI *Insomnia* scores compared to controls. Females scored higher than males on the *Somatic* subscale of the BDI and the *Anxiety* subscale of the DASS. As reported previously, participants with MDD had significantly higher BDI *Total* scores, as well as DEBQ *Emotional* and *Restrained* eating behaviours compared to controls, and females scored higher on each of these scales compared to males. Participants with MDD also had significantly higher total food addiction symptoms compared to controls as indicated by the YFAS. No further effects were identified (Mills et al., 2019).

Table 4.1:
Means and standard deviations for biometric and psychometric data, by Diagnosis and Sex (total $N = 120$; MDD and control participants).

		Diagnosis				Sex			
Variable		MDD M (SD)	Control M (SD)	Effect *p*	Effect Size partial η^2	Female M (SD)	Male M (SD)	Effect *p*	Effect Size partial η^2
	Sample size (*n*)	60	60	-	-	68	52	-	-
	Age (years)	24.70 (6.03)	25.40 (7.17)	.422	.006	24.85 (7.44)	25.31 (5.38)	.709	.001
Biometric	Weight (kg)	74.65 (16.13)	73.29 (16.67)	.721	.001	68.76 (16.18)	80.79 (13.98)*	< .001	.135
	BMI (kg/m^2)	25.80 (5.41)	25.10 (5.35)	.580	.003	25.40 (6.06)	25.51 (4.35)	.910	.000
	Waist Circumference (cm)	89.47 (14.30)	85.36 (12.65)	.149	.018	85.48 (14.74)	89.94 (11.58)	.070	.028
BDI	Total Score	30.72 (9.59)*	4.80 (4.75)	< .001	.753	19.06 (15.88)*	16.06 (13.82)	.029	.040
	Cognitive	18.92 (6.81)*	2.42 (2.95)	< .001	.716	11.38 (10.19)	9.73 (9.28)	.086	.025
	Somatic	11.80 (3.72)*	2.38 (2.50)	< .001	.697	7.68 (6.06)*	6.33 (5.11)	.019	.046
DASS	Depression	24.47 (9.00)*	2.80 (3.45)	< .001	.715	13.71 (13.19)	13.54 (12.46)	.895	.000
	Anxiety	14.77 (8.85)*	2.43 (3.81)	< .001	.456	9.85 (9.50)*	6.96 (8.57)	.020	.045
	Stress	22.37 (9.28)*	5.37 (4.86)	< .001	.573	14.97 (11.43)	12.42 (11.03)	.062	.030
DEBQ	Emotional	2.75 (1.11)*	1.99 (0.72)	< .001	.148	2.60 (.99)*	2.07 (0.95)	.002	.082
	Restrained	2.49 (1.07)*	1.94 (0.83)	.002	.076	2.40 (1.01)*	1.97 (0.93)	.014	.051
	External	3.20 (0.67)	2.98 (0.71)	.117	.021	3.10 (0.69)	3.08 (0.72)	.923	.000
YFAS	Symptom Count	3.27 (3.45)*	0.80 (1.83)	< .001	.160	2.18 (3.12)	1.85 (2.89)	.519	.004
ATQ	Frequency of Thoughts	96.30 (27.5)*	41.55 (13.62)	< .001	.619	72.04 (36.79)	64.85 (32.33)	.069	.028
ISI	Insomnia Score	5.45 (2.30)*	1.88 (1.51)	< .001	.458	3.66 (2.59)	3.67 (2.72)	.975	.000

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index; BDI = Beck's Depression Inventory; DASS = Depression, Anxiety and Stress Scale; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale; ATQ = Automatic Thoughts Questionnaire; ISI = Insomnia Severity Index. *Indicates a significant difference compared to the other diagnostic group or sex being compared.

4.5.1.2 Serotonin

Four (4; 3 MDD, 1 female) univariate outliers were detected in the serotonin data using boxplot diagrams. Further, eleven participants (8 MDD; 10 female) reported insulin dysregulation issues (six with polycystic ovarian syndrome, two with insulin resistance, three with diabetes). The results including all participants (n = 120) and excluding the outliers and those with insulin dysregulation (n = 106) were equivalent in terms of effects and interactions; as such, results from the 120 participants are reported.

Plasma serotonin was significantly higher in participants with MDD than controls ($F(1, 115) = 9.981$, $p = .002$, partial $\eta^2 = .080$), and in males compared to females ($F(1, 115) = 4.323$, $p = .040$, partial $\eta^2 = .036$). Age as a covariate, and the interaction between Diagnosis and Sex, was not significant (Table 4.2).

Table 4.2:
Means and standard deviations for serotonin concentrations (total N = 120; ng/ml), by Diagnosis and Sex (MDD and control participants).

Variable			Serotonin	Main Effect	Effect Size	Interaction
		n	M (SD)	*p*	partial η^2	*p*
Diagnosis	MDD	60	464.17 (269.20)	.002	.080	.772
	Control	60	315.21 (241.55)			
Sex	Female	68	349.01 (240.90)	.040	.036	
	Male	52	441.90 (288.20)			
Covariates	Age	-	-	.117	.021	-

Note: MDD = Major Depressive Disorder.

4.5.2 Analysis by Appetite/Weight Change Sub-Groups in MDD

Following the approach in one of our previous studies (Mills et al., 2019), the 60 MDD participants were sub-classified according to self-reported increases (n = 28) or decreases/no changes (n = 32) to their appetite and weight, based on the DSM-5 criteria ascertained during the MINI clinical interview. The increased group consisted of 11

males and 17 females, whereas the decreased/unchanged group consisted of 15 males and 17 females. Sex distributions did not differ significantly across Appetite/Weight Change groups (χ^2 (1, N = 60) = .350, p = .554), nor did Appetite/Weight Change groups differ significantly in age.

Weight, BMI, DEBQ *Emotional* and *Restrained* eating behaviours and YFAS *Food Addiction* symptoms were significantly higher in participants reporting increased appetite/weight compared to those with decreased/unchanged appetite/weight (Mills et al., 2019). Sex effects for biometric and psychometric data were as reported in section 4.5.1.1. No further effects were identified.

Plasma serotonin levels did not differ significantly between MDD participants reporting increased or decreased/unchanged appetite/weight, nor did values differ significantly by sex in this sub-group analysis. Age was not a significant covariate, and no interaction effect was identified. Means and standard deviations for the biometric, psychometric and serotonin data by Appetite/Weight Change groups are displayed in Table 4.3.

Table 4.3:
Means and standard deviations for biometric, psychometric and serotonin data (total $N = 60$), by Appetite/Weight Categories (increased compared to decreased/unchanged) and Sex (MDD participants only).

		Appetite/Weight Categories				Sex			
Variables		Increased M (SD)	Decreased M (SD)	Effect *p*	Size partial η^2	Female M (SD)	Male M (SD)	Effect *p*	Size partial η^2
	Sample Size (*n*)	28	32	-	-	34	26	-	-
Biometrics	Weight (kg)	78.37 (16.6)*	71.40 (15.23)	.049	0.67	70.58 (14.82)	79.98 (16.48)*	.014	0.104
	BMI (kg/m^2)	27.81 (4.99)*	24.04 (5.20)	.008	.118	26.25 (5.87)	25.21 (4.77)	.580	.006
	Waist Circumference (cm)	93.11 (14.23)	86.28 (13.79)	.072	.056	89.35 (15.39)	89.62 (13.03)	.839	.001
BDI	Total Score	31.96 (10.21)	29.62 (9.02)	.444	.011	33.00 (9.18)*	27.73 (9.44)	.041	.072
	Cognitive	19.50 (7.26)	18.41 (6.47)	.580	.005	20.21 (6.32)	17.23 (7.18)	.117	.043
	Somatic	12.46 (3.85)	11.22 (3.56)	.331	.017	12.79 (3.66)*	10.50 (3.46)	.016	.099
DASS	Depression	25.43 (9.09)	23.62 (8.98)	.561	.006	24.71 (9.72)	24.15 (8.14)	.788	.001
	Anxiety	15.00 (9.47)	14.56 (8.43)	.913	.000	16.65 (8.26)	12.31 (9.15)	.052	.066
	Stress	23.29 (9.51)	21.56 (9.14)	.492	.008	23.94 (8.58)	20.31 (9.91)	.170	.033
DEBQ	Emotional	3.49 (0.98)*	2.10 (0.75)	< .001	.400	3.01 (1.03)*	2.41 (1.12)	.024	.087
	Restrained	2.52 (0.97)	2.47 (1.17)	.964	.000	2.73 (1.07)	2.17 (1.01)	.051	.066
	External	3.35 (0.63)	3.08 (0.69)	.087	.051	3.30 (0.63)	3.10 (0.73)	.447	.010
YFAS	Symptom Count	4.96 (3.74)*	1.78 (2.37)	< .001	.196	3.62 (3.57)	2.81 (3.31)	.415	.012
ATQ	Frequency of Thoughts	103.14 (28.17)	90.31 (25.15)	.101	.047	102.50 (25.06)	88.19 (28.91)	.058	.063
ISI	Insomnia Score	5.54 (2.65)	5.38 (1.99)	.996	.000	5.41 (2.19)	5.50 (2.49)	.996	.000
Serotonin	Serotonin (ng/ml)	457.60 (207.32)	469.93 (316.89)	.837	.001	412.50 (238.06)	531.74 (296.38)	.143	.039

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index; BDI = Beck's Depression Inventory; DASS = Depression, Anxiety and Stress Scale; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale; ATQ = Automatic Thoughts Questionnaire; ISI = Insomnia Severity Index. * Indicates a significant difference compared to the other appetite/weight group or sex being compared.

4.5.3 Correlation Analyses

4.5.3.1 Serotonin Correlations in the Total Sample

Plasma serotonin correlated positively with depressive symptom severity (BDI *Total* score), as well as the cognitive-affective and somatic symptoms (BDI) of MDD. Further, plasma serotonin was positively associated with anxiety-related psychological distress (DASS) and insomnia-related sleep disturbances (ISI). Plasma serotonin was not significantly associated with BMI, depression or stress-related psychological distress (DASS), problematic eating behaviours (DEBQ, YFAS) or depressogenic thinking (ATQ; Table 4.4). Depressive symptom severity (BDI), psychological distress (DASS), negative cognitions (ATQ), sleep disturbances (ISI) and problematic eating (DEBQ, YFAS) were all positively correlated with one another. BMI correlated positively with the DEBQ *Emotional* and *Restrained,* and YFAS, scores (Table 4.4).

FDR-corrected correlations were conducted between plasma serotonin levels and the individual items of the BDI-II to provide a clearer understanding of the relationships between plasma serotonin and depressive symptoms. This analysis found positive associations with eleven items relating to low mood such as pessimism and agitation, negative cognitions such as self-criticism and self-dislike, anhedonia-related symptoms and somatic symptoms such as loss of libido, fatigue and loss of energy (Table 4.5). No further correlations were significant.

Table 4.4:
Pearson's correlation coefficients for the study variables (total *N* = 120; MDD and control participants).

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Serotonin	-												
2. BMI	.003	-											
3. BDI Total Score	.259*	.024	-										
4. BDI Cognitive	.246*	.037	.983*	-									
5. BDI Somatic	.259*	-.001	.950*	.878*	-								
6. DASS Depression	.194	.040	.928*	.929*	.853*	-							
7. DASS Anxiety	.244*	-.050	.816*	.799*	.781*	.755*	-						
8. DASS Stress	.152	.017	.837*	.817*	.806*	.798*	.812*	-					
9. DEBQ Emotional	.017	.274*	.462*	.435*	.473*	.424*	.397*	.465*	-				
10. DEBQ Restrained	-.081	.200*	.335*	.320*	.335*	.343*	.250*	.340*	.375*	-			
11. DEBQ External	.062	-.070	.161	.166	.141	.153	.134	.194	.294*	-.039	-		
12. YFAS Symptoms	.068	.285*	.501*	.471*	.514*	.423*	.372*	.415*	.548*	.216*	.211*	-	
13. ATQ Frequency	.195	.029	.912*	.925*	.817*	.883*	.789*	.793*	.434*	.308*	255*	.480*	-
14. ISI Insomnia	.240*	.078	.712*	.669*	.729*	.660*	.642*	.649*	.322*	.262*	-.016	.394*	.610*

Note: MDD = Major Depressive Disorder; BDI = Beck's Depression Inventory; DASS = Depression, Anxiety and Stress Scale; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale; ATQ = Automatic Thoughts Questionnaire; ISI = Insomnia Severity Index. Effects reported are Benjimani-Hochberg adjusted *p* values (False Discovery Rate corrected); * $\alpha < .05$.

Table 4.5:
Pearson's correlation coefficients for the exploratory analysis of BDI-II items and plasma serotonin (total $N = 120$; MDD and control participants).

BDI-II Item	Pearson's	Effect	BDI-II Item	Pearson's	Effect
	r	*p*		*r*	*p*
1. Sadness	.212	.047*	12. Social Withdrawal	.149	.121
2. Pessimism	.245	.029*	13. Indecisiveness	.177	.081
3. Sense of Failure	.163	.105	14. Sense of Worthlessness	.192	.036
4. Loss of Pleasure	.215	.049*	15. Loss of Energy	.261	.021*
5. Guilt Feelings	.244	.025*	16. Sleep Disturbances	.181	.078
6. Sense of Punishment	.208	.046*	17. Irritability	.161	.105
7. Self-Dislike	.274	.042*	18. Changes in Appetite	.094	.323
8. Self-Criticism	.221	.045*	19. Concentration Difficulties	.140	.139
9. Suicidal Ideation	.160	.100	20. Fatigue	.268	.032*
10. Crying	.073	.427	21. Loss of Libido	.266	.021*
11. Agitation	.203	.049*			

Note: MDD = Major Depressive Disorder; BDI = Beck's Depression Inventory. Effects reported are Benjimani-Hochberg adjusted *p* values (False Discovery Rate corrected); * $\alpha < .05$.

4.5.3.2 Serotonin Correlations by Diagnosis and Sex

The relationships between serotonin levels and the dependent variables were further separately examined in those with MDD and controls, as well as in males and females, to investigate whether serotonin showed diagnostic or sex-based relationships to depressive psychopathology. No significant associations between serotonin and the variables of interest were observed in the MDD or control groups. With respect to sex, in females plasma serotonin levels were significantly associated with the *Agitation* item of the BDI only ($r(68) = .388, p = .021$). In contrast, in males serotonin positively correlated with depressive symptom severity (BDI; $r(52) = .380, p = .018$), cognitive-affective depressive symptoms ($r(52) = .402, p = .033$) and anxiety related psychopathology (DASS; $r(52) = .408, p = .017$). Further, in males plasma serotonin levels were also correlated with the *Punishment* ($r(52) = .462, p = .007$), *Self-Dislike* ($r(52) = .442, p = .011$), *Suicidal Ideation* ($r(52) = .403, p = .013$), *Indecisiveness* ($r(52) = .342, p = .046$), *Worthlessness* ($r(52) = .426, p = .011$) and *Loss of Libido* ($r(52) = .468, p < .001$) items of the BDI. No further correlations were significant.

4.6. Discussion:

This study adds important information to the current understanding of peripheral serotonin concentrations in relation to depressogenic symptoms. Plasma serotonin was significantly higher in individuals with MDD compared to healthy controls, and was also higher in males compared to females. Across the total sample, plasma serotonin correlated positively with depressive symptom severity and insomnia. Serotonin correlated with depressed mood, negative thinking and anxiety in males, but not in females. Plasma serotonin did not differ by appetite and weight symptom presentation in MDD, and was not associated with problematic eating behaviours or physical health indices. These findings support a possible dysregulation of peripheral serotonin in MDD, which is related to select depressive symptoms, particularly in males.

Higher plasma serotonin was observed in individuals with MDD compared to controls, which is consistent with some previous studies reporting elevated plasma serotonin in MDD (Tyano et al., 2006). However, this contrasts with other studies reporting lower or similar plasma serotonin between MDD and control groups, which may be a product of different methodologies and smaller samples used in previous studies (Holck et al., 2019; Pan et al., 2018). Elevated plasma serotonin in MDD is consistent with the hypothesis of upregulation in the peripheral serotonergic system, suggesting differences in serotonergic regulation in individuals with MDD compared to controls (Andrews et al., 2015). The mechanisms underlying plasma serotonergic upregulation remain to be identified, though given the links between plasma serotonin and physical and mental exhaustion, upregulation may reflect compensatory responses to physical and emotional expenditure linked to depressive symptoms in energy balance pathways (Andrews et al., 2015). In addition, elevated peripheral serotonin levels prior to treatment with antidepressant medications has been linked to greater treatment

responsivity (Holck et al., 2019) suggesting that it may be a clinically relevant marker. However, plasma serotonin in MDD remains an under-researched area. Given the inconsistent results in existing literature (Pan et al., 2018; Tyano et al., 2006), longitudinal research is warranted to determine whether upregulation of plasma serotonin is an antecedent or consequence of MDD and its role in treatment outcomes.

A clear sex difference in plasma serotonin concentrations was also observed, with this being higher in males than females. This is inconsistent with limited previous research that identified no difference in plasma serotonin between sexes (Demerdash et al., 2018), however analyses by sex have not been consistently included in previous plasma serotonin studies (Holck et al., 2019; Pan et al., 2018). Higher plasma serotonin in males suggests that plasma serotonin synthesis and regulation may be sexually dimorphic. There was further sexual dimorphism in the correlations between plasma serotonin and depressive symptoms. In males, plasma serotonin was positively correlated with depressive symptom severity, anxiety, cognitive symptoms such as self-dislike, suicidal ideation and feelings of worthlessness and somatic symptoms such as loss of libido, but in females plasma serotonin correlated with agitation only. These associations, in combination with the higher plasma serotonin in males, suggest that serotonin may be relevant to a broader range of depressive symptoms in males compared to females. The sexual dimorphisms identified warrant further research, as plasma serotonin may be important to understanding the aetiology of select depressogenic symptoms, particularly in males, and treatment outcomes, given the links between higher serotonin and antidepressant treatment responsivity (Holck et al., 2019).

Plasma serotonin did not differ significantly between depressed individuals reporting appetite and weight gain compared to loss, nor did plasma serotonin correlate with psychometric indices of problematic eating behaviours or BMI. To our knowledge,

this is the first study to examine these variables in participants with MDD. This is inconsistent with previous research identifying positive associations between plasma serotonin, weight gain and BMI values (Young et al., 2018); however, Young et al. (2018) used a non-depressed and clinically obese sample with a higher average BMI than the current study. The absence of significant relationships between plasma serotonin and problematic eating behaviours in MDD in the current study may be due to greater regulation of the latter by other neuropeptides or hormones such as leptin, ghrelin and peripheral dopamine. Our previous research indicates that these hormones, particularly leptin, were closely related to problematic eating behaviours and weight changes in atypical MDD and in females; however, these hormones were not linked to depressive symptom severity (Mills et al., 2018; Mills et al., 2019; Mills et al., 2020). Despite plasma serotonin not correlating with problematic eating behaviours, plasma serotonin was positively associated with depressive symptom severity and insomnia, as well as with depressogenic thinking in males, which were in turn associated with problematic eating behaviours. Greater depressive symptom severity, sleep disturbances and depressive thinking have previously been linked to a greater risk of problematic eating behaviours (Conradt et al., 2008; Knutson, 2007; Mills et al., 2020), which themselves are risk factors for weight gain and chronic health conditions in MDD (Mills et al., 2019; Mills et al., 2020). These associations, in combination with the sexual dimorphism in plasma serotonin observed in the current study, add to a growing body of research indicating greater neuroendocrine involvement in MDD than previously considered, with peripheral hormones correlating with specific symptom types and sex differences (Mills et al., 2018; Mills et al., 2019; Mills et al., 2020; Thomas & Larkin, 2018; Thomas & Larkin, 2020). The current study suggests that plasma serotonin may be more relevant to males and non-atypical symptom presentations in MDD, as

serotonin was more closely related to cognitive, affective and behavioural depressive symptoms in males, as opposed to being directly connected to an atypical symptom profile featuring depressogenic overeating, weight gain and leptin dysregulation in females (Mills et al., 2019).

Overall, the results from the current study provide new evidence that plasma serotonin is related to select affective, cognitive and behavioural depressive symptoms. These findings also highlight that unlike other peripheral hormones such as leptin, plasma serotonin does not appear to be directly implicated in depressogenic overeating, weight gain and chronic health condition risk as part of atypical MDD. Instead, plasma serotonin may be important to other depressive symptoms including depressive symptom severity, sleep and cognitive disturbances, particularly in males. These findings suggest that upregulation in plasma serotonin may potentially be an important factor in MDD symptoms, particularly in males, and also suggest that hormonal differences between sexes have an important role in symptom presentations in MDD. Further research is needed in order to determine the time course of the identified relationships to further elucidate the role of plasma serotonin in the onset of depressive symptoms and to establish whether plasma serotonin represents an additional or alternative target for MDD treatment. Since peripheral serotonin is more easily measured than CNS serotonin (Andrews et al., 2015), it may have utility as a more accessible biomarker of MDD symptoms and treatment in research and clinical contexts. Given that elevated peripheral serotonin is associated with greater antidepressant responsivity (Holck et al., 2019), if plasma serotonin is higher in males than females with MDD, males in particular may be more responsive to antidepressant medications, which in turn may lead to more tailored interventions by MDD subtype and sex. Further research is warranted to examine this possibility.

Some limitations of the study need to be considered when interpreting the results. The study was limited by its cross-sectional design; longitudinal research is required to further elucidate the pathways between MDD and the onset of depressive symptoms related to plasma serotonin. As central and peripheral serotonin are considered separate pools in the body (Andrews et al., 2015), peripheral serotonin may not be indicative of central serotonergic pathways and functioning; however, evidence in support of permeability of the blood brain barrier, and potential crosstalk between the two pools is emerging (Maurer-Spurej, 2005). Further, given the links between peripheral serotonin and obesity, further research in depressed individuals with higher BMIs may be of interest in order to understand the potential links between MDD and chronic health problems.

To conclude, this study provides new evidence suggesting that plasma serotonin is meaningfully associated with several symptoms of MDD including mood and cognition, but not problematic eating behaviours and weight changes. Longitudinal research is warranted in order to understand the nature of peripheral serotonergic upregulation in MDD, its relationships to the onset of depressive symptoms and to treatment outcomes. This may lead to a greater understanding of the pathophysiology of MDD and to potential alternative treatment targets which may lead to improved outcomes for those affected by MDD.

CHAPTER FIVE

5.1 Introductory Comments

Despite not being directly associated with problematic eating behaviours and physical health indices, Study 3 identified that plasma serotonin was significantly associated with depressive symptoms related to mood, cognition and sleep disturbances, particularly in males. These results provide a preliminary indication that, in contrast to leptin, serotonin may be more relevant to males and a non-atypical presentation of MDD. Further research is needed to examine biological correlates of MDD subtypes, with the findings thus far implying that interventions for MDD and associated chronic health conditions may need to be tailored by MDD symptom presentation and sex.

As observed in Studies 1 and 2, a high proportion of individuals with MDD met the YFAS criteria for food addiction. Dopamine has been implicated in the pathogenesis of addictions, however the role of peripheral dopamine in problematic eating behaviours and food addiction is unknown. Peripheral dopamine is synthesised in the adrenal medulla and is associated with the sympathetic stress response, and may therefore be relevant to food addiction and stress-related overeating behaviours observed in MDD. However, there has been little research conducted to evaluate this hypothesis.

In Study 4, plasma dopamine levels, biometrics and psychometric indices of problematic eating behaviours and low mood were compared between 80 individuals with MDD and 60 healthy controls, in an extended cohort to the previous two studies. A sub-analysis of food addiction group was conducted to investigate whether mood and appetite-related psychopathology and peripheral dopamine levels were related to specific symptoms.

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5.2 Abstract

The concept of food addiction refers to addiction-like behaviours that develop in association with the intake of highly palatable foods. Previous research indicates that a high proportion of individuals with Major Depressive Disorder (MDD) meet the criteria for food addiction, and are also at an increased risk of weight gain and chronic disease. In the central nervous system, dopamine is a neurotransmitter associated with reward salience and food intake, whereas peripheral dopamine is involved in sympathetic stress regulation, digestion and gastrointestinal motility. However, little research has examined relationships between peripheral dopamine, depressive symptoms and problematic eating behaviours in MDD. Biometrics, psychopathology and plasma dopamine levels were compared between participants with MDD (n = 80) and controls (n = 60). Participants were sub-categorised into those meeting or not meeting Yale Food Addiction Scale (YFAS) criteria. Psychometric measures of mood and appetite were used to assess MDD symptoms, problematic eating behaviours and food-addiction related symptoms. Twenty-three (23; 29%) MDD participants met the Yale criteria for food addiction. Depressed individuals meeting YFAS criteria had significantly greater psychopathology scores for both mood and eating compared to depressed individuals not meeting YFAS criteria and controls. A significant interaction between food addiction status and sex was also observed for plasma dopamine levels. Plasma dopamine levels correlated positively with disordered eating behaviours in females, and negatively in males. The results provide evidence that depressogenic excess eating and weight gain are associated with peripheral dopamine levels. Longitudinal research is

warranted investigating endocrine dysregulation and excess eating in MDD, which may inform interventions and reduce chronic disease risk in affected individuals.

Keywords: Peripheral dopamine; Major Depressive Disorder; food addiction

5.3 Introduction

A growing body of evidence indicates that individuals with Major Depressive Disorder (MDD) are at an increased risk of weight gain (Luppino et al., 2010; Mannan et al., 2015). Changes in appetite and weight are symptomatic of MDD, with the direction of such changes varying as a function of MDD subtype (American Psychiatric Association, 2013). Melancholic MDD is associated with appetite and weight loss, whereas atypical MDD is characterised by hyperphagia, weight gain, higher BMI values and a higher prevalence of chronic disease (Cassano & Fava, 2002; American Psychiatric Association, 2013; Lassere et al., 2014). Perhaps due to the increasing prominence of obesogenic environments, depressogenic weight gain as part of atypical MDD is now more prevalent than weight loss and melancholia (Blanco et al., 2012; Privitera et al., 2013). Further, depressogenic weight gain has been observed to occur more frequently in females than in males (Sutin & Zonderman, 2012). Consistent weight gain and carriage of excess weight has been linked to insulin dysregulation, hypertension, sleep apnoea and high cholesterol, which in turn increase the risk of chronic diseases such as cardiovascular disease and metabolic syndrome (Kearns et al., 2014). Consequently, depressed individuals who experience weight gain are at a greater risk of developing these conditions (Zhao et al., 2009; Kearns et al., 2014). Despite the associated increased risk of chronic disease development, the pathways between depressogenic weight gain factors remain unclear, and this is reflected in the lack of integrated treatment approaches that address biological and psychological aspects of

weight gain. An understanding of the mechanisms underlying depressogenic weight gain may lead to improved interventions and treatment outcomes.

A proposed pathway to depressogenic weight gain in atypical MDD is problematic eating associated with changes in complex biopsychosocial pathways. Problematic eating behaviours can be conceptualised into several subtypes. *Emotional eating* is an increase in food intake in response to negative emotions. *Restrained eating* is the deliberate restriction of food intake to encourage weight loss or prevent weight gain, and can be followed by compensatory binge eating behaviours. *External eating* is an increase in food intake in response to sensory food cues, such as the sight and smell of food (van Strien et al., 2016). Depressive symptom severity is associated with greater emotional eating (van Strien et al., 2016; Mills et al., 2019), food restriction (Mills et al., 2019) and food intake in response to external food cues (Sevincer et al., 2017). We previously identified that problematic eating behaviours are more prevalent in MDD than healthy controls, in females than males, and in a subset of depressed individuals experiencing weight gain compared to weight loss. Problematic eating behaviours are also associated with higher BMI and waist circumference values, and related to the hormones leptin and ghrelin (Mills et al., 2019). This evidence suggests that depressed individuals, particularly females, may be at a greater risk of weight gain and chronic disease due to problematic eating behaviours, which are linked to biological factors and may not be purely psychologically driven (Mills et al., 2019).

Food addiction, that is addiction-like behaviours that develop in association with the consistent intake of highly palatable foods high in sugar or fat, is a relatively new approach to understanding problematic eating behaviours (Piccinni et al., 2015). Increased consumption of highly palatable foods has been observed to occur frequently during periods of emotional distress, possibly due to the ability of sugar and fat to

dampen the hypothalamic-pituitary-adrenal (HPA) axis stress response by reducing glucocorticoid sensitivity, thereby reducing arousal and irritability (Dallman et al., 2003) and therefore possibly acting as a coping mechanism for depressed mood (Finch & Tomiyama, 2015). Food addiction has been linked to the catecholamine neurotransmitter dopamine, which is released by both central (CNS) and peripheral (PNS) nervous systems. CNS-dopamine has a core role in the regulation of movement and mood, in addition to the modulation of brain reward and motivation pathways (Drozak & Bryla, 2005; Opmeer et al., 2010). Dopaminergic dysregulation is associated with the formation and maintenance of addictions to psychoactive substances (Volkow et al., 2012), as well as highly palatable foods. With respect to the latter, this can lead to addiction-like behaviours similar to general substance use disorders including a loss of control around particular foods and withdrawal effects when these foods are not consumed (Gearhardt et al., 2009; Gearhardt et al., 2011; Meule & Gearhardt, 2014).

Food addiction is currently not recognised as a formally diagnosable disorder in the Diagnostics and Statistical Manual for Mental Disorders (DSM-5; American Psychiatric Association, 2013), however criteria have been developed to systematically assess it based on the DSM-5 criteria for substance use disorders, including tolerance and withdrawal (Meule & Gearhardt, 2014). Food addiction is currently measured using the Yale Food Addiction Scale (YFAS; Gearhardt et al., 2016), with the prevalence of food addiction as indicated by the YFAS ranging from 5-10% in the general population, and between 15-25% in people with obesity (Meule & Gearhardt, 2014; Hauck et al., 2017).

The concept of food addiction is still controversial since food is considered necessary for survival whereas the targets of other addictions, such as psychoactive substances, are not (Ziauddeen & Fletcher, 2013; Onaolapo & Onaolapo, 2018). It has

been argued that food addiction may be more appropriately defined as 'compulsive overeating', however unlike Binge Eating Disorder (BED) where similar food intake patterns similar to food addiction occur there is no preoccupation with weight, suggesting that they are different syndromes (Burrows et al., 2017). Similarly, food addiction may be more appropriately characterised as 'additive addiction' as the addictive nature of the behaviour is in response to the sugar or fat content of the food, as opposed to the food as a whole (Onaolapo & Onaolapo, 2018). Many modern foods have been altered to increase their palatability compared to natural foods to the extent that they arguably act in similar ways to addictive substances (Leigh & Morris, 2018), which increases the likelihood of consuming these foods in excess (Johnson & Wardle, 2014). As such, food addiction may be a useful approach to understanding depressogenic weight gain in obesogenic environments.

Food addiction has been linked to MDD, with the prevalence rates of meeting YFAS criteria in MDD ranging from 25-28% in some studies (Mills et al., 2018; Mills et al., 2019), which is higher than rates reported in general community samples and among people with obesity (Hauck et al., 2017). Depressive symptom severity has also been linked to higher food addiction symptomology, such as tolerance and cravings (Gearhardt et al., 2012; Mills et al., 2019). Food addiction symptoms are also more common in females overall than in males (Mills et al., 2019). However, beyond its higher prevalence in MDD and its links to depressive symptom severity, depressogenic food addiction remains poorly understood. Due to the relationships between atypical MDD and appetite increases, it is possible that depressogenic overeating may be closely aligned with food addiction, however at present no research has examined this relationship.

The mechanisms underlying depressogenic overeating, including food addiction, also remain unclear; however, dopamine may be related to these behaviours. Activation of CNS-dopamine pathways in response to food is associated with reward and addiction (Gearhardt et al., 2011). Emotional and restrained eating behaviours have been associated with hypoactivity in CNS-dopamine pathways (Volkow et al., 2003; Alsio et al., 2010), and therefore overeating may compensatory to promote dopamine release to enhance reward and improve mood (Avena et al., 2009). CNS-dopamine pathways have also been linked to the pathogenesis of MDD, however findings are inconsistent. Neuroimaging studies indicate lower dopamine receptor binding in individuals with MDD compared to controls, which is linked to the depressogenic symptoms of anhedonia and low motivation (Der-Avakian & Markou, 2012). In contrast, cerebrospinal fluid levels of dopamine have been reported to be higher in MDD compared to controls (Gjerris et al., 1987), whereas a post-mortem study comparing brain concentrations of dopamine following suicides found no difference compared to controls (Bowden et al., 1997).

Peripheral dopamine is more easily measurable in blood, and may be implicated in the context of MDD and overeating; however, there is limited research in this area. In the periphery, dopamine is linked to the homeostatic regulation of blood pressure, respiration, gastrointestinal motility, insulin production, circadian rhythms and the immune response (Rubi & Maechler, 2010). Peripheral dopamine is also closely linked to the HPA axis and stress response, and is positively associated with sympathetic nervous system activity, with higher levels of stress linked to elevated dopamine release (Rubi & Maechler, 2010). Given that HPA axis hyperactivity is a central physiological feature of MDD (Dallman et al., 2003), peripheral dopamine levels may therefore be elevated in MDD. Plasma dopamine levels have been reported to be higher in MDD

compared to controls (Pan et al., 2018), and are also elevated in psychotic MDD compared to non-psychotic MDD (Rothschild et al., 1986). Positive correlations between plasma dopamine levels and psychopathology, including depression, anxiety and stress related symptom severity, have also been reported (Hamner & Diamond, 1996; LeBlanc & Ducharme, 2007; Tomei et al., 2007). However, other studies have reported no difference in plasma dopamine levels between those with MDD and healthy controls (Hamner & Diamond, 1996; Fajardo et al., 2003).

Interestingly, in previous peripheral dopamine studies sex comparisons were not conducted (Fajardo et al., 2003; Pan et al., 2018). Comparing peripheral dopamine levels between males and females would be of interest, particularly due to the higher incidence of food addiction in females (Mills et al., 2019). Peripheral dopamine has also not been examined in relation to food addiction. Further, the role of peripheral dopamine signalling in relation to depressogenic appetite and weight changes has yet to be explored. An understanding of these relationships may provide new information about the nature of depressogenic overeating and weight gain, which may lead to the development of preventative measures to reduce chronic disease risk in those affected by MDD.

The current study aimed to examine the extent of symptoms of overeating, food addiction and plasma dopamine levels in participants with MDD overall compared to controls by sex, in addition to an examination of these symptoms by food addiction status. The current study also aimed to assess the relationships between plasma dopamine, depressive symptoms, problematic eating behaviours and food addiction symptomology.

5.4 Methods

5.4.1 Participants

This study is an extension of previous MDD research (Mills et al., 2019). Participants were recruited by media and university advertisements, with one hundred and forty (140) adults (80 female) included in the study. Individuals reporting depressive symptoms (n = 80) were pre-screened using the Mini International Neuropsychiatric Interview, version 7.0.2 (MINI; Sheehan, 2015) to confirm a current major depressive episode based on DSM-5 criteria. Depressed participants were required to not be receiving any current or recent treatment for MDD. Sixty (60) individuals with no significant mental health history or current diagnosed mental disorders were included in the control group. General exclusion criteria were corticosteroid use, neurological illness and substance use disorders. Participants were asked to provide information about any current medical conditions and medications. The study received approval from the local ethics committee.

5.4.2 Psychometric Measures

Depressive symptom severity was assessed using the 21-item Beck Depression Inventory (BDI-II; Beck et al., 1996). Problematic eating behaviours, including emotional, restrained and external eating, were measured using the 33-item Dutch Eating Behaviours Questionnaire (DEBQ; van Strien et al., 1986). Food addiction symptoms, including withdrawal symptoms and tolerance to increased food intake, were assessed using the 35-item Yale Food Addiction Scale version 2 (Gearhardt et al., 2016).

5.4.3 Procedure

Participants attended one visit at the local Clinical Research and Trials Unit, with appointments scheduled between 9:00 and 11:00am. On arrival, all participants

provided written informed consent. Participants with MDD were interviewed using the MINI (Sheehan, 2015) to confirm a current depressive episode at the time of their visit. Participant height and weight were measured to determine body mass index (BMI) values. Waist circumference, blood pressure and heart rate were also measured. A 10ml blood sample was then obtained by a phlebotomist. Participants were not required to fast, however details of any food intake in the preceding 12 hours were recorded. Participants then completed the psychometric questionnaires. Participants were seated in stable conditions for the duration of the interview and blood sampling in order to reduce possible confounding effects of movement on peripheral dopamine levels.

5.4.4 Data and Statistical Analysis:

Blood samples were centrifuged 4°C, at 3000rpm for 10 minutes immediately following collection, with aliquoted plasma stored at -80°C until analysis. Plasma dopamine analyses were completed using a standard competitive-inhibition ELISA testing kit (Cusabio Technology LLC, Texas, United States of America). The inter- and intra-assay coefficients of the ELISA were 6.8% and 6.4% respectively.

Statistical analyses were conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 25). The dependent variables were plasma dopamine levels, biometrics and the psychometric measurement scores (BDI-II, DEBQ and YFAS). Raw dopamine values were natural-log transformed prior to analyses to better approximate a normal distribution. BDI-II, DEBQ and YFAS scores were also natural-log transformed to correct for skewed distributions, however analyses on transformed and untransformed questionnaire data were equivalent in terms of effects and interactions. As such, results based on untransformed questionnaire data are reported.

Two-way factorial analyses of variance (ANOVA) were used to test for differences in the dependent variables, with the between-subjects factors of Diagnosis

(MDD, control) and Sex (female, male), and Age included as a covariate for dopamine analyses. Two-way ANOVAs were also used to test for subgroup differences in the dependent variables as a function of food addiction status (MDD meeting YFAS criteria, MDD not meeting YFAS criteria, control) and Sex (female, male), with Age also used as a covariate for dopamine analyses. Confirmatory Welch's ANOVAs were conducted when between-group violations in homogeneity of variance were detected in ANOVA analyses, and are reported where appropriate. Pearson's correlation coefficients and Spearman's rank correlations were used to determine relationships between the study variables. An $\alpha < .05$ was considered statistically significant for all analyses. Bonferroni corrected pairwise comparisons are indicated where appropriate.

Using the G*Power statistical tool, version 3.1.9.4, to achieve a statistical power of 80% with significance at $p < .05$, for a factorial ANCOVA with two or three groups, with a medium effect size (.03), a total of 90 participants are required, and for a bivariate correlation with a medium effect size (.03), a total of 84 participants are required. These calculations show that the current study (80 MDD, 60 control) is adequately powered.

5.5. Results

5.5.1 Analyses of MDD compared to Controls

5.5.1.1 Demographic and Biometric Data

Participant demographic and biometric data are presented in Table 5.1. Participants were aged between 18 and 63 years ($M = 25.45$, $SD = 7.16$ years), with 46 females and 34 males in the MDD group ($n = 80$) and 34 females and 26 males in the control group ($n = 60$). Sex distributions did not differ significantly between diagnostic groups (χ^2 (1, $N = 140$) = 0.10, $p = .921$). No participants had a diagnosed eating

disorder, and none were smokers. The diagnostic groups (MDD versus control) did not differ significantly in age.

Participants with MDD had higher waist circumference values than controls (Welch's $F(1, 136.76) = 7.120$, $p = .009$, $\omega^2 = .042$). Males weighed more ($F(1, 136) = 13.183$, $p < .001$, partial $\eta^2 = .088$) and had higher systolic blood pressure (Welch's $F(1, 103.19) = 64.706$, $p < .001$, $\omega^2 = .313$) than females. No further differences based on Diagnosis, Sex or interaction effects were identified for biometric data (Table 5.1).

Table 5.1:
Means and standard deviations for demographic and biometric data, by Diagnosis and Sex (total $N = 140$; MDD and control participants).

		Diagnosis				Sex			
Variable		MDD	Control	Effect	Effect Size	Female	Male	Effect	Effect Size
		M (SD)	M (SD)	*p*	η_p^2/ω^2	M (SD)	M (SD)	*p*	η_p^2/ω^2
	Sample size (*n*)	80	60	-	-	80	60	-	-
Biometric	Age (years)	25 (7)	25 (7)	.880	.000	25 (8)	26 (5)	.595	.002
	Weight (kg)	77.1 (18.1)	73.3 (16.7)	.177	.013	71.6 (18.5)	81.5 (14.6)*	<.001	.088
	BMI (kg/m^2)	26.7 (5.9)	25.1 (5.4)	.173[a]	.014[b]	26.2 (6.6)	25.7 (4.3)	.556[a]	.000[b]
	Waist Measurement (cm)	92 (16)*	85 (13)	.009[a]	.042[b]	88 (13)	91 (12)	.157[a]	.001[b]
	Systolic BP (mmHg)	119 (13)	121 (15)	.581[a]	.000[b]	113 (10)	129 (13)*	<.001[a]	.313[b]
	Diastolic BP (mmHg)	75 (10)	72 (10)	.206	.012	73 (9)	74 (9)	.287	.008

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index. * Indicates a significant difference compared to the other condition(s). [a] Indicates significance for Welch's ANOVA. [b] Indicates ω^2 values.

5.5.1.2 Psychometric Data

Means and standard deviations for psychometric data are presented in Table 5.2. Participants with MDD had significantly greater depressive symptom severity (BDI-II Total score; Welch's $F(1, 119.81) = 418.058$, $p < .001$, $\omega^2 = .749$) compared to controls. The difference between sexes, in addition to the interaction between Diagnosis and Sex, were not significant for the BDI.

Individuals with MDD reported greater DEBQ *Emotional* (Welch's $F(1, 134.06) = 38.807$, $p < .001$, $\omega^2 = .213$), and *Restrained* ($F(1, 136) = 12.766$, $p < .001$, partial $\eta^2 = .086$) eating behaviours than controls. Females had higher *Emotional* (Welch's $F(1, 133.85) = 14.552$, $p < .001$, $\omega^2 = .088$), and *Restrained* ($F(1, 136) = 6.656$, $p = .011$, partial $\eta^2 = .047$) eating scores than males. *External* eating did not differ between diagnostic groups or sexes. No interaction effects were identified for any DEBQ subscales.

Endorsement rates for each YFAS symptom are presented in Table 5.3. Twenty-three (29%, 17 female) MDD participants met the YFAS criteria for food addiction, compared to two (3%, both female) controls. Depressed participants reported higher total YFAS scores (Welch's $F(1, 124.16) = 35.546$, $p < .001$, $\omega^2 = .198$), and also scored higher on each YFAS subscale compared to controls. Females had higher scores on the *Failure to Quit* (Welch's $F(1, 137.06) = 5.127$, $p = .025$, $\omega^2 = .031$) and *Withdrawal* (Welch's $F(1, 136.60) = 5.393$, $p = .022$, $\omega^2 = .031$) subscales compared to males, with no further differences by Sex or interaction effects identified (Table 5.2).

Table 5.2:
Means and standard deviations for psychometric data, by Diagnosis and Sex (total $N = 140$; MDD and control participants).

		Diagnosis				Sex			
Psychometrics		MDD	Control	Effect	Effect Size	Female	Male	Effect	Effect Size
		M (SD)	M (SD)	*p*	η_p^2/ω^2	M (SD)	M (SD)	*p*	η_p^2/ω^2
	Sample size (*n*)	80	60	-	-	80	60	-	-
BDI	Total Score	31 (10)*	5 (5)	< .001[a]	.749[b]	21 (16)	18 (14)	.255[a]	.023[b]
DEBQ	Emotional	3 (1)*	2 (1)	< .001[a]	.213[b]	3 (1)*	2 (1)	< .001[a]	.088[b]
	Restrained	3 (1)*	2 (1)	< .001	.086	3 (1)*	2 (1)	.011	.047
	External	3 (1)	3 (1)	.080	.022	3 (1)	3 (1)	.729	.001
YFAS	Symptom Count	4 (4)*	1 (2)	< .001[a]	.198[b]	3 (4)	2 (3)	.152[a]	.008[b]
	Increased Intake	0.4 (0.5)*	0.1 (0.3)	.001[a]	.072[b]	0.3 (0.4)	0.3 (0.5)	.787[a]	.000[b]
	Failure to Quit	0.4 (0.5)*	0.1 (0.3)	< .001[a]	.126[b]	0.4 (0.5)*	0.2 (0.4)	.025[a]	.029[b]
	Time Taken to Obtain	0.4 (0.5)*	0.1 (0.3)	< .001[a]	.086[b]	0.3 (0.4)	0.2 (0.4)	.821[a]	.000[b]
	Activities Given Up	0.3 (0.5)*	0.0 (0.1)	< .001[a]	.168[b]	0.2 (0.4)	0.1 (0.3)	.158[a]	.007[b]
	Adverse Consequences	0.3 (0.5)*	0.1 (0.3)	< .001[a]	.081[b]	0.2 (0.4)	0.2 (0.4)	.258[a]	.002[b]
	Tolerance	0.3 (0.5)*	0.0 (0.2)	< .001[a]	.140[b]	0.2 (0.4)	0.2 (0.4)	.855[a]	.000[b]
	Withdrawal	0.5 (0.5)*	0.1 (0.3)	< .001[a]	.153[b]	0.4 (0.5)*	0.2 (0.4)	.022[a]	.030[b]
	Use Despite Problems	0.3 (0.5)*	0.1 (0.3)	.001[a]	.072[b]	0.2 (0.4)	0.2 (0.4)	.301[a]	.000[b]
	Failed Role Obligations	0.2 (0.4)*	0.0 (0.1)	< .001[a]	.097[b]	0.2 (.4)	0.1 (0.3)	.152[a]	.007[b]
	Physically Hazardous Use	0.3 (0.5)*	0.1 (0.3)	< .001[a]	.065[b]	0.2 (0.4)	0.1 (0.4)	.670[a]	.000[b]
	Cravings	0.3 (0.4)*	0.1 (0.3)	.002[a]	.055[b]	0.2 (0.4)	0.1 (0.3)	.125[a]	.009[b]

Note: MDD = Major Depressive Disorder; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale. DEBQ scores occur between 0 and 5. YFAS subscale scores are dichotomous and occur between 0.0 and 1.0. *Indicates a significant difference compared to the other diagnostic group or sex being compared. [a] Indicates significance for Welch's ANOVA. [b] Indicates ω^2 values. Significance values for DEBQ and YFAS scores have been corrected for multiplicity.

Table 5.3:
Percentage of endorsement rates for Yale Food Addiction Scale (YFAS) data, by Diagnosis and Sex (total $N = 140$; MDD and control participants).

		Diagnosis		Sex	
		MDD	Control	Female	Male
		%	%	%	%
YFAS	Sample Size (*n*)	80	60	80	60
	Increased Intake	38	13	26	28
	Inability to Quit	41	10	35	18
	Time Taken to Obtain	35	10	25	23
	Activities Given Up	31	2	23	13
	Adverse Consequences	29	7	23	15
	Tolerance	31	3	19	20
	Withdrawal	45	10	38	20
	Use Despite Problems	30	8	24	17
	Failed Role Obligations	21	2	16	8
	Physically Hazardous Use	29	8	21	18
	Cravings	25	7	21	12

Note: MDD = Major Depressive Disorder; YFAS = Yale Food Addiction Scale.

5.5.1.3 Dopamine

Means and standard deviations for the raw and log-transformed dopamine values are displayed in Table 5.4. Following log-transformation, no univariate outliers in the dopamine data were detected in boxplot diagrams. Eleven (11) participants (8 MDD, 3 control; 10 female) reported insulin dysregulation issues (six polycystic ovarian syndrome, two insulin resistance, three diabetes). Log-dopamine values did not differ significantly between those with insulin dysregulation and those without ($F(1, 138) = .950$, $p = .331$), and results for including and excluding these participants are equivalent in terms of effects and interactions. Consequently, dopamine data from the whole sample ($n = 140$) are reported.

Log-dopamine values were marginally higher in MDD compared to controls ($F(1, 135) = 3.820$, $p = .053$, partial $\eta^2 = .028$). Males had significantly higher log-

dopamine values than females ($F(1, 135) = 265.740, p < .001$, partial $\eta^2 = .663$). Age was a significant covariate ($F(1, 135) = 7.488, p = .007$, partial $\eta^2 = .053$). The interaction between Diagnosis and Sex was not significant.

Table 5.4:
Means and standard deviations for raw and log-transformed dopamine (total $N = 140$; ng/ml), by Diagnosis and Sex (MDD and control participants).

Variable			Dopamine	Log-Dopamine	Main Effect	Effect Size	Interaction
		n	M (SD)	M (SD)	*p*	partial η^2	*p*
Diagnosis	MDD	80	29 (35)	3 (1)	.053	.028	
	Control	60	23 (22)	3 (1)			
Sex	Female	80	10 (8)	2 (1)	< .001	.663	.436
	Male	60	48 (35)	4 (1)			
Covariate	Age	-	-	-	.007	.053	-

Note: MDD = Major Depressive Disorder. Significance noted for log-transformed data.

5.5.2 Analysis by Food Addiction Group in MDD

To further investigate depressed individuals with food addiction, all 140 participants were sub-categorised into those with MDD who met the YFAS criteria for food addiction ($n = 23$), those with MDD who did not meet YFAS criteria ($n = 57$) and healthy controls ($n = 58$). Two control participants who met YFAS criteria were removed from these analyses due to the small number in the group. Welch's ANOVAs are reported where violations in homogeneity of variance occurred in conjunction with uneven subgroup sizes. There were 17 females and 6 males in the MDD meeting YFAS criteria group, 29 females and 28 males in the MDD not meeting YFAS criteria group, and 32 females and 26 males in the control group. The sex distributions for the Food Addiction groups were not significantly different (χ^2 $(1, N = 138) = 3.613, p = .164$).

Depressed individuals meeting YFAS criteria had significantly higher weight ($F(2, 132) = 6.102, p = .003$, partial $\eta^2 = .085$), BMI (Welch's $F(2, 60.26) = 8.060, p =$

.001, $\omega^2 = .093$) and waist circumference (Welch's $F(2, 58.25) = 9.575$, $p < .001$, $\omega^2 = .111$) than depressed individuals not meeting YFAS criteria and controls. Sex effects for biometric data are as reported previously (section 3.1.1). No further differences or interaction effects were identified (Table 5.5).

Depressed individuals meeting YFAS criteria reported significantly higher depressive symptom severity (BDI-II *Total*; Welch's $F(2, 51.20) = 266.354$, $p < .001$, $\omega^2 = .794$), emotional (DEBQ *Emotional;* Welch's $F(2, 56.99) = 31.869$, $p < .001$, $\omega^2 = .309$) and restrained (DEBQ *Restrained; F*$(2, 132) = 9.848$, $p < .001$, partial $\eta^2 = .131$) eating behaviours compared to depressed individuals who did not meet YFAS criteria and controls. Depressed individuals meeting YFAS criteria also demonstrated significantly greater total food addiction symptomology (Welch's $F(2, 55.480) = 64.029$, $p < .001$, $\omega^2 = .477$), in addition to scoring significantly higher on each YFAS subscale. Sex differences for psychometric data are as reported previously (section 3.1.2). *External* eating did not differ between Food Addiction groups, and no interaction effects were identified (Table 5.6). Percentage of endorsement rates for each YFAS symptom by Food Addiction group are presented in Table 5.7.

Table 5.5:
Means and standard deviations for biometric data, by Food Addiction Group and Sex (total $N = 138$, MDD and control participants).

Variable		MDD-FA	MDD Non-FA	Control	Effect	Effect Size	Female	Male	Effect	Effect Size
		M (SD)	M (SD)	M (SD)	*p*	η_p^2/ω^2	M (SD)	M (SD)	*p*	η_p^2/ω^2
	Sample size (*n*)	23	57	58	-	-	78	60	-	-
Biometrics	Age (years)	27 (10)	25 (6)	25 (7)	.872	.002	25 (8)	26 (5)	.813	.000
	Weight (kg)	85.4 (18.9)*	74.6 (16.9)	73.6 (16.8)	.003	.085	71.7 (18.5)	81.5 (14.6)*	< .001	.094
	BMI (kg/m^2)	30.3 (5.7)*	25.2 (5.4)	25.1 (5.4)	.001[a]	.093[b]	26.3 (6.7)	25.7 (4.3)	.556[a]	.000[b]
	Waist Circumference (cm)	101 (15)*	88 (14)	85 (13)	< .001[a]	.111[b]	88 (16)	91 (12)	.158[a]	.007[b]
	Systolic BP (mmHg)	118 (13)	120 (13)	121 (15)	.496	.011	113 (10)	129 (13)*	<. 001	.270
	Diastolic BP (mmHg)	78 (10)	73 (9)	72 (10)	.139	.029	73 (9)	74 (9)	.431	.005

Note: MDD = Major Depressive Disorder; FA = Food Addiction; BMI = Body Mass Index; BP = blood pressure. * Indicates a significant difference compared to the other condition(s).

Table 5.6:
Means and standard deviations for psychometric data, by Food Addiction Group and Sex (total $N = 138$, MDD and control participants).

		Food Addiction Diagnosis					Sex			
Psychometric		MDD-FA	MDD Non-FA	Control	Effect	Effect Size	Female	Male	Effect	Effect Size
		M (SD)	M (SD)	M (SD)	*p*	η_p^2/ω^2	M (SD)	M (SD)	*p*	η_p^2/ω^2
	Sample size (*n*)	23	57	58	-	-	78	60	-	-
BDI	Total Score	37 (8)*	28 (9)	4 (4)	< .001[a]	.794[b]	21 (16)	18 (14)	.255[a]	.002[b]
DEBQ	Emotional	4 (1)*	3 (1)	2 (1)	< .001[a]	.309[b]	3 (1)*	2 (1)	< .001[a]	.089[b]
	Restrained	3 (1)*	2 (1)	2 (1)	< .001	.130	2 (1)	2 (1)	.103	.020
	External	3 (1)	3 (1)	3 (1)	.198	.024	3 (1)	3 (1)	.628	.002
YFAS	Symptom Count	7 (3)*	2 (3)	1 (2)	< .001[a]	.477[b]	3 (4)	2 (3)	.152[a]	.008[b]
	Increased Intake	0.6 (0.5)*	0.3 (0.5)	0.1 (0.4)	< .001[a]	.103[b]	0.3 (0.5)	0.3 (0.5)	.787[a]	.000[b]
	Failure to Quit	0.9 (0.3)*	0.2 (0.4)	0.1 (0.3)	< .001[a]	.397[b]	0.4 (0.5)*	0.2 (0.4)	.025[a]	.029[b]
	Time Taken to Obtain	0.7 (0.5)*	0.2 (0.4)	0.1 (0.3)	< .001[a]	.160[b]	0.2 (0.4)	0.2 (0.4)	.821[a]	.000[b]
	Activities Given Up	0.7 (0.5)*	0.2 (0.4)	0.0 (0.1)	< .001[a]	.262[b]	0.2 (0.4)	0.1 (0.3)	.158[a]	.007[b]
	Adverse Consequences	0.8 (0.4)*	0.1 (0.3)	0.1 (0.2)	< .001[a]	.303[b]	0.2 (0.4)	0.2 (0.4)	.258[a]	.002[b]
	Tolerance	0.7 (0.5)*	0.1 (0.4)	0.0 (0.2)	< .001[a]	.273[b]	0.2 (0.4)	0.2 (0.4)	.855[a]	.000[b]
	Withdrawal	0.8 (0.4)*	0.3 (0.5)	0.1 (0.3)	< .001[a]	.329[b]	0.4 (0.5)	0.2 (0.4)	.022[a]	.031[b]
	Use Despite Problems	0.6 (0.5)*	0.2 (0.4)	0.1 (0.3)	< .001[a]	.142[b]	0.2 (0.4)	0.2 (0.4)	.301[a]	.000[b]
	Failed Role Obligations	0.6 (0.5)*	0.1 (0.3)	0.0 (0.1)	< .001[a]	.153[b]	0.2 (0.4)	0.1 (0.3)	.152[a]	.008[b]
	Physically Hazardous Use	0.6 (0.5)*	0.2 (0.4)	0.1 (0.3)	< .001[a]	.120[b]	0.2 (0.4)	0.2 (0.4)	.670[a]	.000[b]
	Cravings	0.6 (0.5)*	0.1 (0.4)	0.0 (0.2)	< .001[a]	.127[b]	0.2 (0.4)	0.1 (0.3)	.125[a]	.010[b]

Note: MDD = Major Depressive Disorder; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale. DEBQ scores occur between 0 and 5. YFAS subscale scores are dichotomous and occur between 0.0 and 1.0. *Indicates a significant difference compared to the other FA group or sex being compared. [a] Indicates significance for Welch's ANOVA. [b] Indicates ω^2 values. Significance values for DEBQ and YFAS scores have been corrected for multiplicity.

Table 5.7:
Percentage of endorsement rates for Yale Food Addiction Scale (YFAS) data, by Food Addiction Group (total *N* = 138; MDD and control participants).

		Food Addiction Categories		
		MDD-FA	MDD Non-FA	Controls
		%	%	%
	Sample Size (*n*)	23	57	58
YFAS	Increased Intake	23	57	58
	Inability to Quit	61	28	14
	Time Taken to Obtain	87	23	9
	Activities Given Up	65	23	9
	Adverse Consequences	70	16	2
	Tolerance	78	9	5
	Withdrawal	74	14	3
	Use Despite Problems	83	30	9
	Failed Role Obligations	61	18	7
	Physically Hazardous Use	57	7	2
	Cravings	57	18	7

Note: MDD = Major Depressive Disorder; FA = Food Addiction; YFAS = Yale Food Addiction Scale.

For peripheral dopamine levels (ng/ml), there was a significant interaction between Food Addiction group and Sex ($F(2, 131) = 4.815$, $p = .010$, partial $\eta^2 = .068$). Bonferroni-corrected simple effects, with an adjusted alpha of .025, indicated that depressed males meeting YFAS criteria ($M = 3$, $SD = 1$) had significantly lower log-dopamine values than depressed males not meeting YFAS criteria ($M = 4$, $SD = 1$) and control males ($M = 4$, $SD = 1$; $F(2, 57) = 4.742$, $p = .012$, partial $\eta^2 = .173$). However, there was no difference in plasma dopamine levels between depressed females meeting YFAS criteria ($M = 2$, $SD = 1$), depressed females not meeting YFAS criteria ($M = 2$, $SD = 1$) and control females ($M = 2$, $SD = 1$; $F(2, 57) = 2.059$, $p = .146$, partial $\eta^2 = .046$). Similarly to previous analyses, log-dopamine values were higher in males than in females ($F(1, 131) = 157.463$, $p < .001$, partial $\eta^2 = .546$), and Age was a significant covariate ($F(1, 131) = 8.790$, $p = .004$, partial $\eta^2 = .063$; Table 8). Log-dopamine levels did not differ significantly across food addiction diagnoses.

Table 5.8:
Means and standard deviations for log-transformed dopamine data (total $N = 138$; ng/ml), by Food Addiction Group and Sex (MDD and control participants).

Variable			Log-Dopamine	Main Effect	G x S Interaction
		n	M (SD)	*p*	*p*
Food Addiction Group	MDD-FA	23	3 (1)		
	MDD-No FA	57	3 (1)	.124	
	Controls	58	3 (1)		.010
Sex	Female	78	2 (1)	< .001	
	Male	60	4 (1)*		
Covariate	Age	-	-	.004	-

Note: MDD = Major Depressive Disorder; FA = Food Addiction; G = Group; S = Sex.
* Indicates a significant difference compared to the other condition(s).

5.5.3 Correlation Analyses

Log-dopamine values correlated positively with weight and systolic blood pressure, and negatively with *Restrained* eating. Weight, BMI and waist circumference each positively correlated with *Emotional* eating and YFAS Symptom Count scores, and BMI also with *Restrained* eating. Waist circumference positively correlated with *Emotional* eating and YFAS Symptom Count. Systolic blood pressure was negatively correlated with BDI Total scores, *Restrained* and *Emotional* eating. Depressive symptom severity (BDI-II), disordered eating (DEBQ) and food addiction (YFAS) correlated positively with one another (Table 9).

Due to the large sex difference observed in previous analyses, relationships between log-dopamine levels, biometric and psychometric data were further examined in males and females separately to assess whether dopamine showed sex-specific relationships to psychopathology. In females, log-dopamine values correlated negatively with age ($r(80) = -.282$, $p = .011$), however were positively correlated with

Emotional ($r(80) = .284, p = .011$) and *Restrained* ($r(80) = .289, p = .009$) eating. Log-dopamine values were also correlated with the *Failure to Quit* (Spearman's $r(80) = .249, p = .026$), *Adverse Consequences* (Spearman's $r(80) = .328, p = .003$), *Withdrawal* (Spearman's $r(80) = .366, p < .001$), *Continued Use Despite Problems* (Spearman's $r(80) = .399, p < .001$) and *Failed Role Obligations* (Spearman's $r(80) = .252, p = .024$) subscales of the YFAS. In contrast, in males log-dopamine values correlated negatively with *Restrained* eating behaviours ($r(60) = -.463, p < .001$).

Table 5.9:
Pearson's correlation coefficients for the study variables (total $N = 140$; MDD and control participants).

Variables	1	2	3	4	5	6	7	8	9	10	11
1. Dopamine	-										
2. Age	-.109	-									
3. Weight	.298**	.262**	-								
4. BMI	.024	.319**	.874**	-							
5. Waist Circumference	.124	.413**	.887**	.896**	-						
6. Systolic BP	.503**	.096	.524**	.276**	.347**	-					
7. Diastolic BP	.103	.235**	.515**	.474**	.504**	.580**	-				
8. BDI Total Score	.014	-.034	.042	.067	.160	-.169*	.055	-			
9. DEBQ Emotional	-.098	.060	.188*	.312**	.277**	-.210*	.017	.493**	-		
10. DEBQ Restrained	-.166*	-.039	.065	.197*	.158	-.190*	-.009	.276**	.350**	-	
11. DEBQ External	.070	.082	-.001	-.016	.005	-.013	-.028	.170*	.325**	-.024	-
12. YFAS Symptoms	.010	.069	.285**	.350**	.367**	-.014	.051	.513**	.567**	.176*	.226**

Note: MDD = Major Depressive Disorder; BMI = Body Mass Index; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire; YFAS = Yale Food Addiction Scale. *$\alpha < .05$, ** $\alpha < .01$.

5.6 Discussion

The current study aimed to provide a greater understanding of depressogenic eating dysregulation and food addiction, and associations with peripheral dopamine levels. It was found that depressed individuals meeting YFAS criteria for food addiction displayed significantly greater mood and eating related psychopathology compared to depressed individuals who did not meet YFAS criteria and controls. Depressogenic food addiction was also associated with a sexual dimorphism in plasma dopamine levels; depressed males meeting YFAS criteria had significantly lower plasma dopamine levels than depressed males not meeting YFAS criteria and male controls, whereas there was no effect of YFAS status on dopamine levels in depressed or control females. Plasma dopamine levels correlated positively with emotional eating and food addiction related behaviours in females, and negatively in males. Plasma dopamine levels were not correlated with mood or other depressive symptoms.

The findings of the current study provide evidence that a high proportion of participants with MDD experience overeating which overlaps closely with the concept of food addiction as measured by the YFAS, characterised by loss of control, tolerance and withdrawal symptoms in relation to food. Problematic eating behaviours were higher in those with MDD overall compared to controls. The proportion of participants with MDD meeting criteria for food addiction in this study was 29%; which is nearly three times higher than food addiction prevalence rates reported in general community samples (Meule & Gearhardt, 2014). This is also similar to our previous findings in MDD in both a separate cohort (Mills et al., 2018) and a smaller, overlapping cohort (Mills et al., 2019) which has been extended for the current study. Across sexes, depressed individuals meeting YFAS criteria demonstrated significantly greater mood psychopathology and problematic eating (emotional and restrained), in addition to

higher health risk indices (BMI and waist circumference), compared to depressed individuals not meeting YFAS criteria and controls. The endorsement of food addiction criteria in a significantly higher proportion of participants with MDD suggests that food addiction, in addition to emotional and restrained eating behaviours, is an important concept which may contribute to risk of weight gain and health problems in MDD. Additionally, the positive correlations between depressive symptom severity, overeating and BMI in the current study suggest that depressogenic overeating may be related to the ability of some types of food to dampen the physiological stress response produced by the HPA axis in order to improve mood (Dallman et al., 2003; Finch & Tomiyama, 2015), which in turn is associated with greater BMI (Mills et al., 2019). Collectively, these findings indicate that food addiction is associated with greater psychopathology and risk for chronic health conditions in MDD.

A significant interaction effect was observed between food addiction group and sex for plasma dopamine levels in individuals with MDD. Depressed males meeting YFAS criteria had significantly lower plasma dopamine levels compared to depressed males not meeting YFAS criteria and control males, whereas plasma dopamine levels did not differ between females by food addiction group. This suggests that plasma dopamine levels are differentially related to depressogenic symptom profiles in males compared to females. This was also reflected in the relationships observed between plasma dopamine levels and problematic eating behaviours. In females, plasma dopamine levels were positively correlated with emotional eating, restrained eating, and select YFAS subscales such as a failure to quit food intake and withdrawal when certain foods are not consumed. In males however, dopamine levels correlated negatively with restrained eating only. These relationships suggest that females with higher dopamine levels, and males with lower dopamine levels, may be more likely to eat excessively in

association with problematic eating behaviours, and also exert a lower degree of control over these behaviours as well. Consistent intake of highly palatable foods in depressed individuals who meet YFAS criteria may increase the risk for weight gain in both sexes (Paans et al., 2018), which is likely related to peripheral dopamine levels. Further research is required to better understand these effects and their significance.

Peripheral dopamine levels were also positively correlated with weight and systolic blood pressure, which is consistent with the role of peripheral dopamine in the regulation of body weight and blood pressure (Rubi & Maechler, 2010). Higher body weight and systolic blood pressure are risk factors for obesity and cardiovascular disease respectively (Kannel, 2000; Kearns et al., 2014). Higher plasma dopamine levels may therefore be related to increased health risks, however further longitudinal research is required to understand whether this is the case.

Dopamine levels were marginally higher in the MDD than control groups. Previous studies are inconsistent, with some previous studies reporting no differences between groups (Hamner & Diamond, 1996; Fajardo et al., 2003), and others reporting elevated dopamine levels in MDD (Rothschild et al., 1986; Pan et al., 2018). Further, in the current study dopamine levels did not correlate significantly with indices of low mood. This is also inconsistent with some previous studies (Hamner & Diamond, 1996; LeBlanc & Ducharme, 2007). These findings suggest that peripheral dopamine may be related to specific symptom subtypes in MDD such as eating patterns, rather than all depressive symptoms; however further research is needed due to the paucity of research.

Collectively, the findings from the current study provide a greater understanding of potential pathways to depressogenic weight gain and chronic disease. Depressed individuals meeting YFAS criteria may be at greater risk of chronic disease linked to greater depressive symptoms, appetite disturbances and altered sympathetic stress

which is related to peripheral dopamine. These results provide further evidence that problematic eating behaviours are common in MDD and are more closely associated with physiological factors than recognised previously (Mills et al., 2019). The role of peripheral dopamine in depressogenic problematic eating and weight gain has at present not been studied extensively. Further research is necessary in order to understand these relationships, which may assist in the development of interventions to reduce chronic disease risk in affected individuals.

Some limitations of the study need to be considered. The current study was cross-sectional in design. Longitudinal research is required to understand the temporal relationships between peripheral dopamine, food addiction and MDD, in order to determine whether these factors may act as potential points of early interventions. The analyses examining food addiction subgroups in MDD should be further investigated in larger samples. Potential covariates such as diet and physical activity levels were also not controlled for. Due to the role of increased consumption of highly palatable food in depressogenic food addiction and weight gain, and the potential moderation effect of exercise and specific dietary habits, such covariates should be considered.

In conclusion, the current study provides novel evidence highlighting the nature of depressogenic food addiction, and its relationships to peripheral dopamine levels. The results indicate that depressogenic overeating, particularly food addiction, may have a greater physiological basis than considered previously and be related to health risk indices associated with greater chronic disease risk. These findings necessitate future longitudinal research to examine the roles of both psychological and physiological factors in depressogenic weight gain, which may lead to improved and integrated interventions.

CHAPTER SIX

Integrated Analyses

6.1 Introductory Comments

The results from Study 4 emphasised the prevalent nature of problematic eating behaviours in MDD, particularly food addiction, with the prevalence of individuals with MDD meeting YFAS criteria identified as 29%. Study 4 also provided the first reported evidence that peripheral dopamine is associated with depressogenic overeating behaviours and biometric indices of obesity. These findings provide additional support that problematic eating in MDD is related to physiological factors, however as this was the first study examining peripheral dopamine in depressogenic overeating, further research is necessary to understand these relationships in more depth.

This thesis has identified that leptin, ghrelin and dopamine are related to depressogenic overeating behaviours and BMI, and that serotonin is related to depressive symptom severity. However, the unique contribution of each hormone to variance in depressive symptoms and health indices has not yet been explored. As such, the unique and collective contributions of leptin, ghrelin, serotonin and dopamine to problematic eating behaviours, depressive symptom severity and biometric indices of health in MDD was investigated. Due to the sex differences identified in the proportion of problematic eating behaviours and hormones levels in all thesis studies, regression analyses with Sex as a moderating variable were performed.

6.2 Data and Statistical Analysis

The data for the integrated analyses were derived from MDD and control participants in Studies 2, 3 and 4 in whose plasma all four biomarkers had been analysed (n = 140; 80 female). The variables of interest with respect to hormones were leptin (normalised to waist circumference), ghrelin, serotonin and dopamine levels. BMI

was included as an index of physical health. The psychometric variables were depressive symptom severity (BDI total score), problematic eating behaviours (DEBQ *Emotional* eating score) and food addiction (YFAS symptom count).

Pearson's and Spearman's rank correlation coefficients were used to assess interrelationships between Sex, peripheral hormones, health indices and psychopathology. The false discovery rate (FDR) procedure (Benjamini & Hochberg, 1995) was applied to control for Type I errors. Four hierarchical multiple regressions were conducted to investigate the potential moderating effects of Sex on the relationship between hormones and BMI, depressive symptom severity, emotional eating and food addiction respectively. For all four regressions, Sex was entered in the first step, and values for all four hormones (leptin normalised to waist circumference, ghrelin, serotonin and dopamine) entered in the second step. Interaction effects between Sex and each hormone were entered as the third step. Interaction terms were calculated by standardising hormone values to prevent multicollinearity and multiplying these values by Sex (female = 0, male = 1). The visual inspection of plots, tolerance values greater than 0.10 and variance inflation factor values below 2 indicated that the assumptions of normality, linearity, multicollinearity and homoscedasticity were satisfied.

6.3 Correlation Analyses

The correlation results for the variables of interest are reported in Table 6.1. Leptin positively correlated with BMI, problematic eating behaviours (DEBQ, YFAS) and Sex, and negatively correlated with dopamine. Ghrelin negatively correlated with BMI only. Serotonin correlated positively with dopamine and depressive symptom severity (BDI), and negatively with Sex. Dopamine also negatively correlated with Sex. Sex positively correlated with *Emotional* eating. Further, depressive symptom severity, emotional eating, food addiction and BMI were positively correlated with one another.

Table 6.1:
Correlation coefficients for the study variables (total $N = 140$).

Variables	1	2	3	4	5	6	7	8
1. Leptin	-							
2. Ghrelin	-.136	-						
3. Serotonin	-.094	-.021	-					
4. Dopamine	-.436*	-.107	.178*	-				
5. BMI	.493*	-.330*	-.084	.033	-			
6. BDI Total	.136	-.094	.207*	-.009	.067	-		
7. DEBQ Emotional	.408*	-.153	-.064	-.104	.312*	.493*	-	
8. YFAS Symptoms	.191*	-.151	-.007	.003	.350*	.513*	.567*	
9. Sex[a]	.611*	.060	-.209*	-.788*	-.042	.088	.316	.069

Note: BMI = Body Mass Index; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire, YFAS = Yale Food Addiction Scale. Effects reported are False Discovery Rate corrected p values. * $p < .05$. [a] Spearman's correlation coefficients.

6.4 Hierarchical Multiple Regression Analyses

The results of the four hierarchical regressions are reported in Table 6.2. In the first analysis investigating the influence of Sex and peripheral hormones on BMI, in Step 1, Sex accounted for a non-significant 0.2% of unique variance in BMI ($F(1, 138) = .309, p = .579, R^2 = .002$). In Step 2, the addition of the hormone values accounted for an additional 38.9% of unique variance in BMI ($F(5, 134) = 17.248, p < .001, R^2 = .389$). At this step, higher leptin and lower ghrelin were related to significantly higher BMI values. At Step 3, the interaction between Sex and hormones accounted for a further 15% of unique variance in BMI ($F(9, 130) = 17.052, p < .001, R^2 = .150$). Only the relationship between leptin levels and BMI was significantly moderated by Sex. Post-hoc analyses using the procedure outlined in Holmbeck (2002) were conducted to determine the nature of the interaction. Male centred (male = 0, female = 1) and female centred (female = 0, male = -1) Sex variables were multiplied by standardised leptin values to create new interaction terms. Two regression analyses including the main effect for leptin, one of the Sex variables and the interaction term were then conducted. The results of these analyses indicated that higher leptin levels were associated with

greater BMI values across sexes, however this effect was more pronounced in females ($\beta = 1.327, p < .001$) than males ($\beta = .352, p < .001$).

In the second analysis relating to depressive symptom severity, at Step 1 Sex explained a non-significant 0.9% of unique variance in BDI *Total* scores ($F(1, 138) = 1.262, p = .263, R^2 = .009$). At Step 2, the four hormone values significantly explained an additional 7.5% of unique variance ($F(5, 134) = 2.468, p = .036, R^2 = .075$), where higher plasma serotonin values were the only predictor of higher BDI *Total* scores. At Step 3, the interactions between Sex and hormones were not associated with depressive symptom severity, explaining a non-significant 1.5% of unique variance in BDI *Total* scores ($F(9, 130) = 1.578, p = .126, R^2 = .015$).

In the third analysis pertaining to emotional eating, Sex at Step 1 accounted for a significant 9.3% of unique variance in DEBQ *Emotional* eating scores ($F(1, 138) = 14.083, p < .001, R^2 = .093$). The four hormones at Step 2 explained a further 13.3% of unique variance ($F(5, 134) = 7.795, p < .001, R^2 = .133$), with higher levels of leptin and dopamine associated with higher *Emotional* eating scores. At Step 3, the overall regression model including interaction effects was significant and accounted for an additional 3% of unique variance in *Emotional* eating scores ($F(9, 130) = 4.946, p < .001, R^2 = .030$), however only main effects for Sex and leptin levels were observed, and no interaction terms between Sex and the hormones were significant.

In the final analysis assessing food addiction symptoms, in Step 1 Sex explained a non-significant 1.4% of variance ($F(1, 138) = 1.947, p = .165, R^2 = .014$), and in Step 2, the four hormones explained an additional non-significant 5.8% of variance ($F(5, 134) = 2.088, p = .071, R^2 = .058$). Further, at Step 3 the interaction terms between Sex and hormone values explained a non-significant 3% of variance in YFAS *Symptom Count* scores ($F(9, 130) = 1.652, p = .107, R^2 = .003$).

Table 6.2:
Standardised and unstandardised regression coefficients for the hierarchical regression models (total $N = 140$).

		BMI			BDI *Total*			DEBQ *Emotional*			YFAS *Symptom Count*		
		B	β	*p*	*B*	β	*p*	*B*	β	*p*	*B*	β	*p*
Step 1	Sex	.544	.047	.579	2.908	.095	.263	.676	.304	<.001*	.767	.118	.165
Step 2	Leptin	1.806	.651	<.001*	.649	.088	.403	.165	.309	.002*	224	.143	.179
	Ghrelin	-.953	-.214	.003*	-.917	-.077	.365	-.090	-.105	.182	-.310	-.123	.154
	Serotonin	-.002	-.095	.168	.013	.227	.008*	.000	-.023	.772	.000	.007	.939
	Dopamine	.658	.117	.290	2.115	.141	.297	.350	.321	.011*	.715	.224	.101
Step 3	Leptin x Sex	5.235	.506	<.001*	2.572	.094	.442	.186	.093	.401	.221	.038	.755
	Ghrelin x Sex	-.385	-.054	.600	-2.289	-.121	.404	-.337	-.246	.064	-1.074	-.267	.067
	Serotonin x Sex	-1.205	-.146	.084	-.904	-.041	.726	.040	.025	.815	-.285	-.061	.604
	Dopamine x Sex	1.518	.161	.199	-2.866	-.115	.514	-.077	-.042	.792	.331	.062	.722

Note: BMI = Body Mass Index; BDI = Beck's Depression Inventory; DEBQ = Dutch Eating Behaviours Questionnaire, YFAS = Yale Food Addiction Scale.

6.5 Discussion

The results from the integrated analyses provide further evidence that problematic eating behaviours, depressive symptoms and indices of adiposity in MDD are associated with peripheral hormones. Higher leptin and lower ghrelin concentrations were significant predictors of BMI, with a stronger effect between leptin and BMI identified for females compared to males. Across sexes, higher leptin and dopamine levels were significant predictors of *Emotional* eating, and higher serotonin concentrations predicted more severe depressive symptoms. In contrast, when including all hormones in the regression analyses, no hormone accounted for unique variance in the number of food addiction symptoms. These findings suggest that individuals with higher leptin and dopamine levels may experience greater instances of overeating behaviours and weight gain, and that individuals with higher serotonin levels may experience more severe depressive symptoms.

In combination, these findings provide evidence that hormones related to satiety and energy balance account for unique variance in depressive symptoms, which supports a potential and specific role of peripheral hormones in depressogenic symptomology. Broadly, leptin and dopamine levels may act as risk factors for overeating, and serotonin levels may act as risk factors for more severe depressive symptoms. Consistent with the sexual dimorphisms observed in leptin, serotonin and dopamine levels in Studies 2, 3 and 4, females may be at greater risk of overeating behaviours linked to leptin and dopamine levels, whereas males with higher serotonin levels may be at greater risk of more severe depressive symptoms. Further, the relationship between peripheral hormones and adiposity indices differed by sex, with higher leptin levels acting as a potential risk factor for greater weight gain in females compared to males. These differences highlight that sex differences in MDD symptom

presentation and hormone levels are an important consideration with respect to depressogenic weight gain. Taken together, these findings support that the assessment of these peripheral hormones may assist in providing important information about depressive symptom risk, particularly weight gain.

However, these findings are preliminary in nature and should be interpreted tentatively. While these results are promising, larger longitudinal studies are needed in order to provide more detailed information regarding these findings.

CHAPTER SEVEN

General Discussion

The aims of this doctoral thesis were to systematically assess the nature of depressogenic problematic eating behaviours, and to examine their relationship to peripheral hormones, health risk indices and depressive symptom profiles in order to better understand the associations between MDD, obesity and cardiometabolic disease. Concerning these aims, four empirical investigations were conducted utilising an integrated biopsychosocial approach. Problematic eating behaviours, peripheral hormones implicated in the regulation of satiety, hunger, weight, energy balance and the sympathetic stress response, biometric health indices and depressive symptoms were compared between those with MDD and unaffected controls, between females and males, and by symptom profile of depressogenic appetite and weight changes. The interrelationships between these variables were also assessed. The results of this thesis indicate that there is a high prevalence of problematic eating behaviours in MDD, and that these are associated with greater BMI and waist circumference values, as well as the peripheral hormones leptin, ghrelin and dopamine, particularly in females. Serotonin was associated with depressive symptom severity, particularly in males. This thesis highlights possible pathways to weight gain, obesity and cardiometabolic disease risk in individuals affected by MDD, particularly in females.

6.1 Summary of Findings

The first study of this thesis assessed whether depressive appetite/weight symptom profiles, problematic eating behaviours and physical health indices were related to leptin. Leptin concentrations were associated with sex-specific appetite/weight change patterns and problematic eating behaviours in MDD. Leptin was higher in females with appetite/weight gain and in males with appetite/weight loss.

Problematic eating behaviours, including emotional eating and food addiction, were more prevalent in females than males with MDD, and correlated positively with leptin concentrations. Leptin also correlated with greater physical health indices, such as BMI. The findings from Study 1 suggest that higher concentrations of plasma leptin may have an important role in appetite/weight changes and overeating behaviours in MDD, particularly an atypical MDD symptom profile and in females, providing a preliminary indication that problematic eating and risk factors for chronic health conditions in MDD are related to hormonal factors.

Study 2 expanded on the previous study by evaluating whether problematic eating behaviours differed between those with MDD and healthy controls by sex, as well as by appetite/weight symptom profile. Associations between problematic eating in MDD and leptin, the hunger hormone ghrelin, and physical health indicators such as BMI were also assessed. It was identified that problematic eating behaviours occur frequently in MDD and vary by sex and symptom profile. Emotional eating and food addiction were higher in MDD than in controls, particularly in those with self-reported appetite/weight gain, and more prevalent among females. Leptin was positively, and ghrelin was negatively, associated with emotional and restrained eating and food addiction, as well as BMI. These findings from Study 2 highlight the high prevalence of overeating in MDD, and provide additional evidence that these behaviours and physical health indicators are associated with hunger and satiety hormones.

Having established links between problematic eating and hunger and satiety hormones, Study 3 investigated peripheral serotonin, an energy balance hormone, in MDD compared to controls, by sex and appetite/weight symptom profile. The relationships between serotonin, problematic eating and other depressive symptoms were also assessed. Upregulation in serotonin was identified as a potentially important

factor in depressive symptomology, with varying effects across sexes. Serotonin levels were elevated in MDD and in males. Serotonin correlated positively with depressive symptom severity, negative thinking and anxiety in the total sample and in males, but linked to agitation only in females. Serotonin was not associated with problematic eating or weight changes. Study 3 highlights that, unlike the associations observed with leptin and atypical MDD profiles in females, plasma serotonin may be more relevant to non-atypical MDD symptom profiles and males with MDD, further indicating that sex differences in peripheral hormones may be important to depressive symptom presentations.

Following the high prevalence of problematic eating behaviours in MDD identified in the previous studies, Study 4 investigated the role of peripheral dopamine in depressogenic overeating and food addiction in greater detail. It was identified that depressed individuals who met YFAS criteria for food addiction may be at greater risk of obesity and cardiometabolic disease linked to depressive symptoms and altered sympathetic stress responses. Measures of psychopathology, overeating behaviours and indices of health risk including BMI and waist circumference were greater in individuals with MDD who met YFAS food addiction criteria than those with MDD who did not meet YFAS criteria and controls. Plasma dopamine correlated positively with overeating in females and negatively in males, providing additional support that problematic eating in MDD is related to hormonal factors, and that sex differences are an important factor in these relationships.

The final study of this thesis investigated the individual and combined contributions of leptin, ghrelin, serotonin and dopamine to variance in problematic eating behaviours, depressive symptom severity and BMI, and the potential moderating effect of Sex on these relationships. The findings of this study support the differential

roles of peripheral hormones in depressive symptoms, as leptin and dopamine accounted for unique variance in problematic eating behaviours and serotonin accounted for unique variance in depressive symptom severity. Leptin also accounted for a significant quantity of variance in BMI, particularly in females. Study 5 provides additional support for the role of peripheral hormones in depressive symptoms, with the sex-based difference in adiposity, as indexed by BMI, linked to leptin levels. These findings, in combination with the sex-based differences in overeating and peripheral hormone patterns observed in the previous thesis studies, indicate that MDD symptom presentations and peripheral hormones should be considered with respect to the risk of depressogenic weight gain, particularly in females.

6.2 Collective Implications

This thesis has made several novel contributions to the knowledge and literature regarding the nature of and potential mechanisms related to problematic eating in MDD by systematically assessing the relationships between problematic eating behaviours, biometric health indices, depressive symptom profiles relating to appetite and weight, and peripheral hormones. This in turn provides novel information regarding the associations between MDD, obesity and cardiometabolic disease risk. As a result of this research program, several important conclusions about these relationships can be made.

The results of the studies contained within this thesis indicate that there are distinct overeating patterns that occur in MDD relative to unaffected controls, which vary as a function of sex and depressive symptom profile. In all studies, emotional and restrained eating behaviours, as well as food addiction symptoms, were consistently higher in those with MDD compared to controls, in females overall compared to males, and in females with MDD compared to males with MDD. Problematic eating behaviours were also higher in individuals reporting increased appetite and weight

during a depressive episode and in individuals with MDD who met the YFAS criteria for food addiction. These patterns are consistent with the atypical subtype of MDD, which is more prevalent in females and is characterised by hyperphagia and weight gain during a depressive episode (American Psychiatric Association, 2013). The present research was also the first to identify prevalence rates of food addiction in MDD, of 24-29% (Mills et al., 2018; Mills et al., 2019; Mills et al., 2020), which is much higher than the current prevalence rates reported in obese or control populations (Meule & Gearhardt, 2014). Despite the high prevalence rates observed in the present thesis, the underlying biopsychosocial motivations for problematic eating in MDD remain to be elucidated. It has been suggested that problematic eating behaviours may be used by individuals with MDD in order to improve mood (Finch & Tomiyama, 2015). However, emerging evidence suggests that mood improving effects associated with highly palatable food intake are transient (Finch et al., 2019), and that problematic eating behaviours may therefore be used to mitigate physiological stress; including HPA axis activity which is elevated in MDD (Dallman et al., 2003; Keller et al., 2016). Interpreting the current results in the broader context of previous research, the increased emotional eating observed in those with MDD, including females and those with an atypical symptom profile, may be reflective of greater attempts to improve mood or physiological stress during a depressive episode (Finch & Tomiyama, 2015; Finch et al., 2019), which is supported by the positive correlations between problematic eating behaviours, depressive symptom severity and psychological stress in Studies 2, 3 and 4. The potentially transient nature of the mood improving effects may contribute to the repeated and excessive food intake experienced in MDD (Finch et al., 2019). These effects may reinforce highly palatable food intake patterns in some individuals, which may eventually manifest as food addiction (Martins et al., 2019). Alternatively,

hormonal changes such as increased leptin or dopamine concentrations may lead to changes in hunger and associated eating behaviours. Such changes overlap with several types of problematic eating, such as food addiction, as observed in Study 4. The greater restrained eating observed in those with MDD may be indicative of increased binge eating following caloric restriction, or attempts to correct changes to weight following such binges (van Strien et al., 2016). The fact that external eating did not differ across groups in any study indicates that interoceptive hunger signals such as peripheral hormones relating to hunger and satiety, rather than environmentally derived food intake signals, may predominate in MDD. This contrasts with the increased sensory food intake that occurs in obesogenic environments in individuals unaffected by MDD (Hidaka, 2012). Collectively, these findings contribute to the understanding of the prevalence of overeating patterns in MDD, with the greater instances of emotional eating, restrained eating and food addiction observed in Studies 1-4 suggesting that these problematic eating behaviours related to mood and possibly to stress-relief, particularly food addiction, may be a more common occurrence in MDD than previously recognised. In addition to those with MDD, females and those specifically with an atypical MDD symptom profile characterised by overeating and weight gain may be more prone to engaging in these behaviours.

Despite the popular perception that problematic eating behaviours are psychologically motivated (Finch & Tomiyama, 2015), this thesis highlights that peripheral hormones related to hunger, satiety, weight, energy balance and sympathetic stress are related to depressogenic overeating. Leptin levels were positively associated with emotional eating, restrained eating and food addiction, and were significant predictors of problematic eating behaviours across sexes and adiposity particularly in females. Due to the usual role of leptin in satiety, positive associations between leptin

and overeating are consistent with an interpretation of leptin resistance or insensitivity to the expected satiety cues (Pan et al., 2014). If this was the case, higher leptin levels, particularly in females and those with MDD reporting increased appetite and weight, were not signalling the expected satiety response, resulting in increased appetite and increased food intake, particularly emotional eating. This may have in turn influenced weight gain risk due to the relationship between food intake and weight, in addition to the role of leptin in promoting adipogenesis to store excess energy following consistent overeating (Maffei et al., 1995). It is also possible that due to its moderating effect on HPA axis activity, leptin may be implicated as a signalling molecule for emotional eating in order to promote stress relief (Roubos et al., 2013).

Ghrelin levels were negatively associated with restrained eating only, which may indicate increased ghrelin secretion. Due to the roles of ghrelin in hunger (Wren et al., 2000) and promoting adipogenesis during times of insufficient energy to ensure adequate energy stores (Tschop et al., 2001), individuals with higher ghrelin levels may have been experiencing greater hunger and therefore increased food intake. This may have resulted in dieting related behaviours and a greater vulnerability to compensatory binge eating, which may influence weight gain risk.

The correlations between plasma dopamine and problematic eating behaviours varied by sex, as plasma dopamine correlated positively with emotional eating and food addiction, and predicted problematic eating behaviours, in females only. In contrast, plasma dopamine negatively correlated with restrained eating in males only. Further, plasma dopamine predicted *Emotional* eating across sexes. These relationships suggest that peripheral dopamine may be implicated in the differential regulation of problematic eating in males and females. Similarly to leptin, it is possible that plasma dopamine may act as a signalling molecule for highly palatable food intake as part of emotional eating

and food addiction in order to promote stress relief, due to its links to the sympathetic stress response (Rubi & Maechler, 2010). As a result, females with higher and males with lower plasma dopamine levels may have been more likely to overeat and have a lower degree of control over food intake. However, the link between plasma dopamine and eating-related behaviours has not been previously reported in the literature and warrants further research.

In contrast to leptin, ghrelin and dopamine, serotonin was not significantly associated with problematic eating behaviours or physical health indices. However, serotonin accounted for unique variance in depressive symptom severity across sexes, and was positively correlated with other depressive symptoms related to mood, depressogenic thinking and insomnia in males and agitation in females. While plasma serotonin has been previously linked to higher BMI values and obesity (Young et al., 2018), it is possible that plasma serotonin may have a greater role in broader psychopathology in MDD, including symptom severity, with effects varying by sex. Similarly to dopamine, the link between plasma serotonin and depressive symptoms warrants further research to understand the nature of the identified associations.

Taken together, the associations observed in this research indicate that depressogenic problematic eating behaviours are related to several hormone measures. Previous research has indicated that MDD is associated with neuroendocrine changes, particularly in relation to cortisol and HPA axis activity (Keller et al., 2016; Thomas & Larkin, 2018; 2020). This thesis provides new indications of further neuroendocrine involvement in MDD which has meaningful associations with specific symptoms, particularly problematic eating behaviours. As such, the risk for depressogenic weight gain, obesity and cardiometabolic disease may be linked to neuroendocrine changes in hunger, satiety, weight regulation and energy balance hormones, particularly leptin.

Further, the fact that leptin predicted greater indices of adiposity in females, that leptin and dopamine were associated with overeating in females, and that plasma serotonin predicted depressive symptom severity in males supports the importance of peripheral hormones in depressive symptom patterns which vary by sex. The associations observed across all studies of this thesis suggest that upregulation in leptin, ghrelin and plasma dopaminergic pathways, along with hormone resistance or insensitivity, in MDD may be risk factors for depressogenic overeating and weight gain in females, and upregulation in serotonin may be a risk factor for more severe depressive symptoms in males, however further research is required to assess the temporal nature of these relationships.

This thesis has contributed to the understanding of the physiological and psychological factors which may explain depressogenic overeating and associated health risks by identifying significant associations between problematic eating behaviours, peripheral hormones and indices of obesity and cardiometabolic disease risk. Previous studies have considered the individual contributions of problematic eating behaviours and hormones to weight gain in MDD, however interrelationships had not yet been assessed (Milaneschi et al., 2017a; Paans et al., 2018). In the cohorts in the current research program, emotional eating, restrained eating and food addiction symptoms were associated with greater BMI and waist circumference values. Leptin and dopamine were positively, and ghrelin negatively, associated with indices of adiposity, whereas serotonin was not related to overeating or adiposity measures. These relationships provide new information that problematic eating behaviours and distinct peripheral hormone profiles may act as potential risk factors for depressogenic weight gain, with overweight-obesity risk varying by sex. Weight gain and obesity are known risk factors for additional health complications, which compound the risk for chronic

disease onset including obesity and cardiometabolic disease (Zhao et al., 2009; Kearns et al., 2014). As such, problematic eating behaviours and peripheral hormones may need to be carefully considered in the treatment of depressogenic weight gain. Importantly, this thesis also indicates that sex differences in these factors need to be carefully considered, as the greater prevalence of overeating and associated hormonal patterns in females and absence of such patterns in males may have important implications in that interventions for these issues may need to vary by sex (Smith et al., 2008).

The interrelationships between problematic eating behaviours, hormones and indices of obesity suggest that integrated biopsychosocial approaches may show promise in future approaches to addressing depressogenic weight gain. There is currently a lack of holistic approaches to understanding weight gain in MDD as existing perspectives are predominantly either psychological or physiological in nature (Arora & Anubhuti, 2006; Paans et al., 2018). This is reflected in existing intervention methods oriented on either therapy for underlying emotional issues or a perceived lack of willpower, or generic weight loss approaches such as dieting, exercise or pharmacotherapy regimes (Jacka & Berk, 2012). However, these perspectives are limiting as potential interactions between variables may be overlooked. This research program has shown that an integrated approach incorporating biological, psychological and behavioural measures provides new information which can potentially increase the understanding of the risks of additional health burdens in affected individuals. Further integrated research is needed to better understand depressogenic overeating, which may lead to novel integrated interventions to assist in the prevention of this issue and associated health risks.

Given the high prevalence of problematic eating in MDD, and the observed associations with indices of obesity in this research program, problematic eating

behaviours and peripheral hormones may represent potential targets for integrated treatment approaches. From a practical perspective, a biopsychosocial approach to address depressogenic weight gain may consist of the development of a multidisciplinary health team to assess the specific psychological and physiological symptoms that an individual with MDD presents with, and subsequently tailoring therapeutic and behavioural interventions for the individual based on their symptom and behavioural profile. Such interventions may include a combination of tailored cognitive-behavioural therapy, exercise or addressing eating behaviours with a dietician. A pharmacological element may also be included to address hormonal changes that occur in association with problematic eating behaviours and weight gain (Abizaid et al., 2006). Further, given their greater susceptibility to MDD and adiposity (Blaak et al., 2001; Rainville et al., 2018; Smith et al., 2008), females may also require different or more tailored interventions compared to males. The results of this thesis have provided further information indicating that overeating and select hormone patterns are more prevalent in females, which may be relevant to treatments for atypical MDD. Atypical MDD, characterised by appetite and weight gain, is more prevalent in females (Woelfer et al., 2019). As such, females may need to be more carefully assessed if they present with depressive symptoms related to weight gain risk, such as problematic eating behaviours. Leptin insensitivity could be assessed as a potential risk factor for weight gain in females, and leptin and dopamine levels in females could also be assessed as potential biomarkers of risk for problematic eating behaviours. Such assessments may lead to early preventative measures to reduce obesity and cardiometabolic disease risk in affected individuals, however, as this is one of the first programs of research to assess the interrelationships between problematic eating behaviours, hormones and weight gain in MDD, more research is required to assess the nature of the identified

relationships in more depth. Given that the prevalence rates of MDD and chronic disease are increasing annually (World Health Organisation, 2017), this area of research is a priority in order to assist in reducing the likelihood of chronic disease risk in affected individuals.

6.3 Thesis Limitations

The findings from this thesis should be viewed in light of some methodological limitations. Firstly, cross-sectional study designs were utilised in all four studies, therefore causation cannot be inferred from the identified results and an understanding of the temporal nature of the identified relationships is limited. For example, it is not clear whether weight gain causes changes in peripheral hormones, or whether peripheral hormones affect appetite and eating behaviours, which can then cause weight gain. As such, longitudinal research is warranted to provide more specific indications about the directionality of the identified pathways between problematic eating behaviours, biological factors and weight in MDD, which will assist in further elucidating the pathways between MDD, obesity and cardiometabolic disease risk.

Secondly, the sub-group analyses in Studies 2 to 4 were conducted in small groups of participants, and despite promising results from these investigations with respect to MDD symptom profile and sex differences in problematic eating behaviours, peripheral hormones and physical health indicators, these findings should be considered preliminary in nature and therefore interpreted tentatively. Further research replicating these analyses in larger groups will provide greater statistical power and may provide a clearer indication of the relationships between the variables of interest.

Thirdly, participants were not required to fast prior to blood collection. Previous studies have indicated that fasting levels of leptin and ghrelin are not significantly different to non-fasting levels (Hancox & Landhuis, 2011; Natalucci et al., 2005),

whereas fasting differences in peripheral serotonin and dopamine have not been reported. In the thesis studies, no significant differences were observed in any peripheral hormone levels between participants who elected to fast and those who did not. Given the natural endocrine fluctuations in response to hunger and satiety, further research utilising fasting protocols may provide more detailed information regarding endocrine changes in the context of problematic eating in MDD.

Further, several potential confounding variables such as participant diet, physical activity levels and current subjective hunger levels were not accounted for. Because of the role of dietary preferences and subjective hunger levels in eating behaviour, and the moderating influence of exercise on body weight, such covariates should be considered in future research. While measures of increased and decreased appetite were included in all studies, hunger scales may be useful in future studies to provide a quantitative index of hunger levels. In addition, data about menstrual cycle was not collected; menstrual cycle phase in females should also be considered in future research to account for any potential interactions between monthly fluctuations in female hormones, such as oestrogen, with the peripheral hormones of interest.

6.4 Directions for Future Research

Following the outcomes of the studies included in this research program, longitudinal research is warranted in order to understand the nature of the identified associations in more depth. A longitudinal study has commenced within the research group which aims to address the aforementioned limitations and include further variables. Additional survey measures have been included to measure physical activity levels and obtain a subjective rating of hunger. A semi-structured diet history interview, conducted by a dietician, has also been included to measure participant diet and food

intake preferences. It is anticipated that this new study will assist in providing a clearer understanding of the pathways between MDD and chronic disease risk.

In addition to replicating the studies conducted in this thesis, further studies should examine the possible role of other biological factors in problematic eating behaviours and weight gain, in order to provide a more thorough understanding of the relationship between MDD and chronic disease. Cortisol may be of interest due to its close associations with HPA axis activity, energy metabolism and food intake (George et al., 2010). Insulin and neuropeptide-Y may also be of interest due to their roles in the regulation of hunger and satiety (Loh et al., 2017; Steiner et al., 2019). Oxytocin may also be relevant due to its roles in stress attenuation and learning, particularly in the context of addiction (Sarnyai & Kovacs, 2014). Pro-inflammatory cytokines such as tumour-necrosis-factor alpha (TNF-α) and interleukin-6 (IL6) should also be considered due to the role of inflammation in the onset and maintenance of chronic health conditions such as cardiovascular disease (Shajib & Khan, 2015). Similarly, the role of genetic factors in weight gain and MDD may assist in elucidating these relationships (Milaneschi et al., 2017b). Due to the potential role of physiological stress in problematic eating behaviours, future studies would benefit from including additional measures of physiological arousal, such as skin conductance or electroencephalography, in order to assess the relationships between physiological stress indices and overeating. Further, despite the difficulties in quantifying hormone levels directly in the brain of living humans (Andrews et al., 2015), it may be of interest for future research to evaluate CNS measures of these hormones using CSF or neuroimaging techniques and compare them to peripheral levels in blood. Such studies may provide a better understanding of the relationships between the central and peripheral roles of these hormones in MDD, particularly in relation to obesity and cardiometabolic disease risk.

6.5 Concluding Remarks

To conclude, this thesis aimed to systematically investigate problematic eating behaviours and specific peripheral hormones, with a view of better understanding depressogenic weight gain and associations between MDD, obesity and cardiometabolic disease risk. It was identified that there is a high incidence of problematic eating behaviours, particularly food addiction, in those with MDD and in females. Differences in levels of hormones related to hunger, satiety, weight regulation, energy balance and the sympathetic stress response which aligned with the patterns of problematic eating behaviours were also observed; particularly leptin and dopamine in females. Despite the popular perceptions of 'comfort eating', these findings suggest that problematic eating behaviours in MDD are not purely psychological as they are associated with physiological factors. Peripheral hormone levels, particularly leptin, may have potential utility in acting as risk factors for problematic eating behaviours and weight gain in those affected by MDD, particularly females. This leads to the suggestion that integrated models and treatment perspectives be applied to depressogenic overeating and weight gain, which may lead to the development of more integrated and effective interventions in order to reduce the risk of obesity and cardiometabolic conditions in affected individuals. It is hoped that this program of research, and future research extending from it, may help address the increased risk of chronic health conditions in individuals affected by MDD.

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"It's like a game of chess – it's one move at a time, and bit by bit everyone makes a contribution. Even if you do 10%, it's something."

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